

**The Environmental Protection Agency's White Paper  
on Bt Plant-pesticide Resistance Management**

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## Preface

The Environmental Protection Agency's White paper on Bt Plant-pesticide Resistance Management was originally prepared as a support document for the February 9-10, 1998 Office of Pesticide Programs (OPP) Federal Insecticide, Fungicide and Rodenticide (FIFRA) Science Advisory Panel Meeting (SAP) on *Bacillus thuringiensis* (Bt) plant-pesticides resistance management. Written statements and other background documents for this SAP meeting can be obtained from the OPP Docket Office, 703-305-5805, Docket Number, OPP-00231. The EPA White Paper can also be obtained electronically from the EPA Home Page at: Federal Register--Environmental Documents--"Law and Regulations" (<http://www.epa.gov/fedrgstr/>).

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## Introduction: Background and Purpose of the Paper

The Environmental Protection Agency (EPA) has considered the development of pesticide resistance and pesticide resistance management in its regulatory decisions (see reviews Matten *et al.*, 1996 and updated in Matten, 1997). In general, pesticide resistance management is likely to benefit the American public by reducing the total pesticide burden on the environment, and by reducing the overall human and environmental exposure to pesticides. Although EPA does not yet have a published policy or standard data requirements in place for pesticide resistance management, it has required the submission of such data on a case-by-case basis. EPA supports the efforts of all stakeholders to promote pesticide resistance management through the development and use of pesticide resistance management plans, appropriate pesticide labeling and education programs. It is the desire of EPA that this focus on pesticide resistance management not overly burden the regulated community, jeopardize the registration of reduced risk pesticides, or exclude conventional pesticides or other control practices which can contribute to the further adoption of integrated pest management (IPM). EPA believes that appropriate resistance management can further these goals. EPA is continuing to evaluate and refine the role pesticide resistance management has in the Agency's regulatory decisions.

With a greater focus on pollution prevention and pesticide risk reduction, the EPA believes that it is important to implement effective resistance management strategies for pesticides such as *Bacillus thuringiensis* (Bt) plant-pesticides. A great deal of Agency attention has focused on the potential development of resistance to the delta-endotoxins of Bt genetically-engineered into plants (Bt plant-pesticides). This is because Bt plant-pesticides produce the pesticidal active ingredient, the Cry delta endotoxin, throughout the growing season. Long-term exposure to a pesticide is one of the factors increasing the potential selection pressure upon both the target pests and any other susceptible insects feeding on the transformed crop. EPA recognizes the value of Bt plant-pesticides as effective and safer pest management tools and has determined it is appropriate to conserve this resource by requiring resistance management plans for certain transformed crops. The Agency has reviewed the initial strategies from registrants for managing resistance to Bt delta endotoxins produced in potato (Bt potato), field corn (Bt corn), and cotton (Bt cotton) and, when necessary, made certain recommendations and requirements for the development of data to develop and implement long-term resistance management strategies as part of the registration decisions. EPA has worked and is working with stakeholders (industry, university and USDA extension entomologists, individual growers, user groups, trade organization, public interest groups, and government agencies) to address resistance management to Bt plant-pesticides.

The purpose of this paper is to analyze data generated in the 1996 growing season for current resistance management plans for *Bacillus thuringiensis* plant-pesticides for Bt potato, Bt corn and Bt cotton, identify technical modifications that might improve approaches to resistance management, identify areas of ongoing research, and determine what might be required in the future for successful implementation of long-term (sustainable) resistance management for Bt plant-pesticides in these and other crops. This analysis will include information presented in the

two public hearings hosted by EPA in March and May of 1997, 1996 growing season reports on resistance management activities and 1997 research efforts for Bt potato, Bt corn, and Bt cotton, published literature, information from public meetings and discussions with academic or extension entomologists, EPA reviews of the initial resistance management strategies, and EPA FACT sheets.

This paper will analyze progress made in resolving issues related to the appropriate resistance management plans for Bt crops. Good resistance management is dependent on multiple tactics to decrease the selection pressure on the target pest(s) and employment of different mortality sources. For Bt plant-pesticides, as for conventional pesticides, an overall IPM program should include pest resistance management. The characteristics of plant-pesticides (i.e., production and use in the plant) allow the implementation of unique pest management strategies. An example for Bt plant-pesticides is the use of a high dose expression strategy coupled to the use of an effective refuge as important resistance management tools. For all pesticides, an effective resistance management plan is likely to include appropriate predictive tactics, scouting, sampling, and monitoring for changes in pest susceptibility, and evaluation measures to determine the success of the plan. Perhaps, most critical to the success of a resistance management strategy, is communication and education efforts targeting growers to understand and implement the resistance management strategy.

High dosage expression of genes encoding pesticidal proteins will theoretically eliminate all but rare homozygous resistant individuals. The expectation is that 100 percent of the susceptible genotypes will be killed by the high dose of the pesticide. Homozygous recessive (i.e., resistant) individuals are assumed to be so rare as to be insignificant. Effective refuges allow survival of sufficient numbers of susceptible homozygous individuals to maximize the probability that resistant homozygotes will mate with susceptible homozygotes, producing heterozygous progeny that cannot survive on the Bt crop. While the theory of high dose expression coupled to effective structured refuge is relatively straightforward, its implementation has been controversial. That is, there is disagreement as to what is the necessary arrangement and relative size of Bt and refuge field plots, the nature and objective of performance-monitoring activities, and appropriate incentives to foster grower education and acceptance. The Agency has and continues to foster efforts to resolve these disagreements to the satisfaction of all stakeholders and has offered opportunities for public comment and participation.

No field resistance to Bt plant-pesticides has occurred since the first registrations were issued in 1995. Field resistance to Bt microbial sprays exists for a number of geographically-isolated diamondback moth populations worldwide: U.S. (Hawaii, Florida, New York), Asia (China, Japan, Malaysia, Thailand, the Philippines), and Central American (Costa Rica, Guatemala, Honduras, and Nicaragua) (reviewed in Liu and Tabashnik, 1997; Perez and Shelton, 1997). Resistance to Bt has also been detected for *Plodia interpunctella* (Hübner) in stored grain (McGaughey and Beeman, 1988). Bt resistance in several insects has been reviewed by Tabashnik (1994a) and Bauer (1995). Reports of diamondback moth resistance to Bt microbial pesticides have increased everyone's awareness that insect resistance management is important

not just for conventional pesticides, but for biologically-based pesticides, including Bt microbial pesticides and Bt plant-pesticides.

At the time of writing this paper, the EPA has registered six Bt plant-pesticide active ingredients and the genetic material needed for their production in potato, field corn, and cotton (seven registered products) with certain resistance management recommendations and requirements. Four of the six plant-pesticides registered for full-scale commercial use have been for the CryI(A)b or CryI(A)c Bt delta endotoxin and its respective genetic material necessary for its production in field corn to control European corn borer (ECB). The six registered Bt plant-pesticides are as follows: (1) Cry IIIA delta endotoxin and the genetic material necessary for its production in potato (Bt potato) to control Colorado potato beetle (CPB) (registered May 1995); (2) Cry 1(A)b delta endotoxin (and pCIB4431) and the genetic material necessary for its production in corn (Event 176-derived hybrids, Bt corn) to control European corn borer (ECB) (2 products registered August 1995); (3) Cry1A(c) delta endotoxin and the genetic material necessary for its production in cotton (Bt cotton) to primarily control tobacco budworm and pink bollworm, but also to control cotton bollworm (registered October 1995); (4) Cry1(A)b delta endotoxin and the genetic material necessary for its production in corn (and pZ01502; BT11-derived hybrids) to control ECB (registered August 1996); (5) Cry1(A)b delta endotoxin (and pV-ZMCT01; MON 801- and MON 810-derived hybrids) and the genetic material necessary for its production in corn to control ECB (registered December 1996); and (6) Cry1(A)c delta endotoxin (plus three different plasmids) and the genetic material necessary for its production in corn (DBT418-derived hybrids) to control ECB (registered March 1997).

The Agency mandated specific resistance management data requirements and mitigation measures with resistance management strategy for all of the Bt corn and Bt cotton registrations. These registrations were conditional registrations to allow for completion of the studies related to resistance management. Collection of various data, e.g., target pest biology and behavior, secondary pest biology and behavior, population dynamics, cross-resistance potential, refuge strategies, dose deployment adequacy, discriminating concentration, monitoring, and reporting were made conditions of registration for the Bt corn and Bt cotton registrations. Refuge requirements were mandatory for Bt cotton. Development of a draft refuge strategy by August 1998 and a final refuge strategy by January 1999 was required of Bt corn registrations. No requirements related to resistance management were imposed on the registration of CryIIIA delta endotoxin in potatoes based on the Agency's analysis and comments received from the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Science Advisory Panel (SAP) Subpanel on Plant-Pesticides which met on March 1, 1995 (see Office of Pesticide Program (OPP) docket, OPP-00401). Voluntary interaction between the registrant and EPA was recommended by the SAP and certain areas of research and monitoring were suggested. However, Monsanto/Naturemark requires a mandatory refuge through their Grower's Agreement for each of its growers to follow and the overall Bt potato resistance management strategy is being refined as more data become available.

The registration for CryIIIA  $\delta$ -endotoxin in potato is not time-limited (no expiration date).

Registrations for the CryI(A)b  $\delta$ -endotoxin in Event 176-derived corn hybrids, MON 810-derived corn hybrids, BT 11-derived corn hybrids, and CryI(A)c  $\delta$ -endotoxin in DBT 418-derived corn hybrids expire on April 1, 2001. Registration for the CryI(A)c  $\delta$ -endotoxin expressed in cotton expires January 1, 2001. EPA will reevaluate the effectiveness of each registrant's resistance management plan for Bt corn and Bt cotton as more data become available.

This paper will be organized into five basic sections: (I.) General discussion of public hearing issues, (II.) Bt potato resistance management, (III.) Bt corn resistance management, and (IV.) Bt cotton resistance management, and (V.) Conclusions.

## **I. General Discussion of Public Hearing Issues**

EPA continues to monitor and participate in development of resistance management plans for Bt potato, Bt corn, and Bt cotton. Because of the high degree of public interest in effective resistance management for Bt plant-pesticides, the Agency held two public hearings, March 21, 1997 in Washington D. C. and May 21, 1997 in College Station, TX to collect information on the resistance management plans for plant-pesticides. EPA requested comment on four issues: (1) The requirement for resistance management plans; (2) Scientific needs for resistance management plans; (3) The use of "Public Good" as a criterion for the requirement of resistance management plans; and (4) Performance of Bt cotton.

Approximately 100 individuals/organizations submitted written comments and/or delivered presentations regarding the subject of Bt plant-pesticide resistance management and the four issues open for comment. Approximately 30 presenters provided comments at the March 21, 1997 public hearing and approximately 10 presenters provided comments at the May 21, 1997 public hearing. The total number of comments can be classified as follows: industry and seed companies (7), industry-related groups and trade organizations (3), national/state grower organizations (6), growers (9), entomologists from academic institutions and the U.S. Department of Agriculture (extension, research, and forest service) (24), private citizens (31), public advocacy groups (12), Entomological Society of America (ESA) (1), U.S. Congress (1), Canadian Pest Management Regulatory Agency (1), and State FIFRA Issues Research and Evaluation Group (SFIREG) Working Committee on Pesticide Operations and Management (1). A general analysis of the comments will be provided in this section. It should be noted that individuals did not necessarily comment on all four issues. Copies of the written comments are available in the Office of Pesticide Programs public docket, OPP-00470. Part I of this paper presents information EPA received at the hearings. Bt cotton performance will be specifically examined in Part IV of this paper. Specific scientific data needs for development of long-term resistance management strategies will be discussed within the context of specific issues related to Bt potato (Part II), Bt corn (Part III), and Bt cotton (Part IV).

### **A. The requirement for resistance management plans**

The Agency sought comment on whether resistance management plans should be mandatory (i.e.,



required as a term and condition of registration) or voluntary. EPA also sought information on the criteria that would indicate a need to require a resistance management plan. All individuals in one fashion or another indicate that the fundamental basis for resistance management is responsible product stewardship. Based on the verbal presentations and written comments, there was a split response as to whether the Agency should allow companies to voluntarily institute resistance management plans or mandatorily impose such plans as a condition of registration as a means for ensuring successful implementation of Bt plant-pesticide resistance management. In terms of the numbers of actual individuals, there was about a 75:25 split in favor of mandatorily requiring resistance management plans.

Individuals and organizations representing environmental groups, state grower associations, private citizens, organic farmers, USDA Forestry Service, ESA, SFIREG, National Cotton Council, Texas Corn Producers Board, USDA extension and research, and academia (with one exception) all supported EPA mandatorily requiring resistance management plans to ensure the long-term success of the resistance management for Bt plant-pesticides. In fact, all of the private citizens, the organic farmers, organic growers associations, and environmental groups urged EPA to specifically: 1) Suspend current registrations and forego future approvals of Bt crops until workable resistance management plans are available. 2) Convene a meeting of the Scientific Advisory Panel to evaluate the current management plans. 3) Make resistance management plans mandatory. The Union of Concerned Scientists modified the first point and recommended that EPA: “require 40 to 50% non-sprayed Bt-cotton refuges in Bt-cotton fields in the 1997 growing season or suspend the current registration of Bt cotton until workable resistance management plans are available.” These individuals all stated, with some urgency, the desire to maintain the efficacy of Bt products and thus maintain the environmental benefits of Bt plant-pesticides and Bt foliar pesticide products. The basic sentiment expressed was that durability of Bt plant-pesticides is too important to be left to depend on voluntary programs.

Particularly noteworthy in recommending a mandatory role for the Agency was Dr. Mark Whalon, entomologist, Michigan State University, for the protection of “susceptibility genes” through pest resistance management. He made three recommendations. First, the EPA should require resistance management plans for all newly registered conventional or biological pesticides “which will be sold into markets where target or non-target insects, mites, nematodes or pathogens have developed resistance in the past.” In particular, he noted that resistance management plans are important to have in markets where chronic resistance problems exist. Second, the Agency should be required to have “independent scrutiny of the enforcement of susceptibility management (refuge) in the case of cotton and corn Bt-transgenic plants.” He believes that the corn situation poses a greater scientific risk than the cotton situation. Third, EPA should convene a Science Advisory Panel to advise the Agency of “the development of a comprehensive Susceptibility Management Assessment process in the Agency’s pesticide registration responsibility.”

SFIREG commented that it shares a common goal with EPA in wanting resistance management strategies that prevent resistance from developing. “Both the states and EPA understand that

overuse of a single pesticide will likely lead to the development of resistant pests and eliminate the utility of that tool.” A unified product stewardship/education program by registrants for both the Bt plant-pesticides and Bt microbial pesticides does not exist. In light of that, SFIREG advised EPA to take a “cautious” regulatory approach and mandate resistance management plans. SFIREG urged EPA to use requirements on the product registration itself to manage resistance. The states do not believe that use of labels as an alternative approach to resistance management would be effective because currently there are many unresolved issues with plant-pesticide labeling, especially as related to enforceability.

Researchers from Texas A & M commented that “the use of refugia of a minimal size relative to the acreage of transgenic plants in the area, in our opinion, should be mandatory.” They further comment that as more scientific information becomes available the size of the refuge may be increased or decreased, as appropriate. Other comments from this same group indicated that resistance monitoring is critical and should be done by non-company sources such as Departments of Entomology in collaboration with USDA or other government agencies. EPA should be a funding agency for basic research, grower education, and monitoring programs, but not make these areas a requirement for registration because of the difficulties in enforcing these requirements.

Conversely, individuals from industry (except for Praxis), seed companies, the American Seed Trade Association, Insecticide Resistance Action Committee, two members of Congress from Idaho [Mike Crapo (U.S. House of Representatives) and Larry Craig (U.S. Senate)] and individual cotton farmers indicated that EPA should not establish “additional hurdles” to the development and implementation of these new Bt plant-pesticides and that it is industry’s (including seed company’s) responsibility to ensure the successful development and implementation of resistance management strategies. That is, resistance management to Bt plant-pesticides should be handled on a voluntary basis. The individual cotton farmers (2) who commented indicated that Monsanto’s actions with regard to developing and implementing a resistance management program were very useful to help preserve the durability of Bt cotton. “These actions not only made good business sense for Monsanto, but for all cotton growers as well.” In general, opinions expressed indicated that resistance management for Bt plant-pesticides was industry’s responsibility and that EPA’s role should be to evaluate the safety of pesticidal products and to participate in discussions with growers, academicians, government scientists (especially USDA extension and research scientists) and industry to work together to make resistance management work. EPA should not make specific resistance management requirements nor require additional data to support particular resistance management strategies development because of the dynamic nature of pest management, cropping practices, and other factors in which a great deal of flexibility is required for their implementation. Market forces should dictate resistance management strategies. EPA’s role should not be one of enforcement of resistance management plans.

A number of individuals, in particular, from industry, Biotechnology Industry Organization (BIO), American Crop Protection Association (ACPA), and seed companies stated that there should be a

“level playing field” for resistance management of Bt plant-pesticides. These comments can be summarized by Monsanto’s statement regarding implementation of Best Management Practices (BMPs) that there should be “a collaborative stewardship process which should not require direction by the Agency, but one in which all stakeholders including the EPA participate and take ownership.” Monsanto described a multiple stakeholder collaboration that would result in the development of the elements of BMPs for specific Bt plant-pesticides. Monsanto’s current view of appropriate BMPs include the following: “binding growers to follow insect resistance management BMPs (e.g. grower agreements), immediate implementation of refuge, extensive grower education programs, surveillance for and grower reporting of suspected resistance (e.g. 1-800 telephone numbers), and mitigation planning to address resistance development.” Unlike Monsanto, other industry individuals (Novartis, Mycogen, Pioneer Hibred International, and Holden Foundation Seeds) did not endorse nor describe specific BAMPs in their comments. BIO supported a collaborative multi-stakeholder effort. Flexibility in the development and implementation of specific resistance management strategies was cited as the primary reason for a non-mandatory role for EPA by a number of individuals. These individuals argued that agroecosystems are dynamic and flexibility is necessary to respond to dynamic conditions. BIO specifically noted that currently each individual company must develop its own resistance management strategy and research priorities and this is not necessarily cost-effective. Several individuals from industry and academia noted, as an example of a multi-stakeholder approach to developing resistance management strategies, the coordinating efforts of the USDA NC-205 (the regional project entitled “Ecology and Management of the European Corn Borer and Other Stalk-Boring Lepidoptera”) that brings together representatives from academia, government, industry (developers of the gene technology and seed producers), and consultants to discuss and develop European corn borer (*Ostrinia nubilalis* Huebner, ECB) resistance management strategies for Bt corn and grower education materials.

Some individuals from industry and academia/USDA indicated that EPA should not single out Bt plant-pesticides for resistance management requirements. As the statement from Pioneer Hibred International notes “EPA must be equitable in imposing data requirements; it should not single out plant-pesticides for requirements that have not been, and are not being, imposed on numerous other registrants of Bt pesticides.” Other individuals noted that the Agency did not impose resistance management requirements on conventional chemicals that posed a high degree of selection pressure for resistance on the same target insects as Bt plant-pesticides, e.g., imidacloprid, spinosad, fipronil, and insect growth regulators. Pioneer Hibred International suggested that if a mandatory role was necessary for implementation of resistance management strategies it should be USDA rather than EPA that should have the lead.

The USDA Forest Service expressed concern about the potential for development of insect resistance to Bt used to control forestry pests as a result of registration and use of Bt plant-pesticides. Currently no Bt plant-pesticides are registered for forestry use. The Forest Service recommends that EPA fully assess and document the potential effect of Bt plant-pesticides in accelerating forest insect resistance, collect the necessary scientific information to develop resistance management plans and require these plans to be implemented to manage forest insect

resistance to Bt plant-pesticides.

## **B. Scientific information needed for resistance management plans**

EPA sought comment on the scientific information needed to develop effective resistance management plans. That is, what kinds of data are necessary to assess the potential for pest resistance and/or adequately evaluate proposed resistance management plans. Most specific comments came from university or USDA entomologists and industry. However, all individuals indicated a need to collect more scientific information related to resistance management.

Representatives from industry (in particular, Novartis Seeds, Mycogen, Monsanto, and Pioneer) believed that areas where more research is needed have been clearly identified and that research projects were underway to address these areas. Academic and USDA researchers are active participants in a number of these research projects. There were comments received on the research needed for development of sustainable resistance management strategies for Bt potato, Bt corn, and Bt cotton. The areas identified were: pest biology (including movement (larval and adult, mating behavior)), ecology (including host range of target and secondary pests especially in the Bt corn and Bt cotton agroecosystems), behavioral responses such as emergence differences between resistant and susceptible pests (including examination of relative fitness differences), susceptibility of the pest(s) to the insecticide in question, differences in population dynamics, baseline susceptibility and development of discriminating dose assays for the target pests, species-specific resistance models (development and validation), monitoring for the development of insect resistance including the development of molecular probes for early detection of the evolution of resistance in the field, ensuring an adequate high dose, estimates of initial resistance gene frequencies, impact on beneficial insects, use of alternate hosts as refuges, effective refuge strategies (development and validation), and development of hybrid varieties that utilize native resistance genes or other novel resistance genes that can be stacked or pyramided with existing Bt genes. Research projects in these areas will be discussed more specifically in the context of particular resistance management strategies for Bt potato (Section II), Bt corn (Section III), and Bt cotton (Section IV) below.

Drs. John Witkowski and Blair Siegfried (corn entomologists), University of Nebraska-Lincoln, in their comments to EPA, identified specific research questions and their importance in developing resistance management plans. For example, these researchers commented that quantifying ECB local movements and gene flow is critical to determining effective refuge sizes that maximize the probability that susceptible individuals from a structured refuge will find and mate with the few resistant individuals anticipated to survive exposure to the Bt plant. Additionally, they stated that it is desirable to understand the biochemical and physiological nature of resistance in the target pest(s), cross-resistance patterns, and inheritance of resistance. Such questions can be addressed by selecting for Bt-resistant ECB populations in the laboratory. Dr. Liebe Cavalieri (State University of New York at Purchase) commented on the value of predictive models in understanding the insect pest-pathogen interaction as a basis for the design of experiments that can be carried out in laboratory microcosms.

Several cotton entomologists, as noted below, also provided comments regarding research efforts and strategies to develop long-term resistance management strategies. These same basic research areas identified above for Bt corn exist for the target lepidopteran pests (tobacco budworm, pink bollworm, and cotton bollworm) in the Bt cotton agroecosystem. Several researchers stressed the importance of using Bt cotton in the context of IPM, especially the use of crop rotation. That is, the use of the Bt plant-pesticide technology is just one element in the total mix of pest control strategies in an IPM program.

Texas A & M researchers provided extensive comment on 5 research areas, primarily for Bt cotton, but also for other Bt crops:

1. Assess area wide resistance monitoring needs. [e.g., collect baseline susceptibility data for major and minor/secondary pests; set up monitoring programs, determine patterns of cross-resistance in field populations (including use of simulations)]
2. Assess the number of users of *Bacillus thuringiensis* (Bt) foliar pesticide products vs. plant-pesticides using an area wide survey approach.
3. Seek information on toxin expression in transgenic plants. [e.g., public disclosure of Bt toxin expression patterns throughout the plant for the full growing season; ensure high dose expression (i.e., ensure killing of most heterozygotes, assume that resistance is partially recessive and due to a single gene)]
4. Seek information on dispersal from refuge to transgenic crops and vice-versa. [e.g., adult movement, larval movement]
5. Assess the effect of synthetic pyrethroid insecticide treatments against cotton bollworm on Bt cotton and on the effectiveness of the refuge.

Texas A & M researchers commented that evaluation of any proposed resistance management plan should include the following two points: 1) EPA could assess compliance with refuge adoption, preference and management plan and 2) EPA could request data to determine the relationship between the level of insect survival on a Bt crop and the size of refuge needed. They also indicated that requesting resistance management plans only for Bt plant-pesticides and not other pesticides is “scientifically and logically flawed”.

Dr. William Meredith (cotton entomologist), USDA/ARS/CP&G in Stoneville, Mississippi recommends the following to investigate in-field success of Bt cotton performance:

1. Investigate in the field various management strategies, not just one strategy. Data from many grower groups in the Mississippi Delta could provide a valuable data base to determine which resistance management parameters are important and do further studies.

2. Establish a public monitoring system that follows the various management strategies.
3. Establish a public data base collected from the various strategies.
4. Evaluate the effectiveness of various strategies by a team of researchers. Final data evaluation should be made public.

Possible management parameters would include: appropriate use of chemical and biological insecticides, crop rotation, rotation of non-Bt and Bt cotton varieties from year to year, develop and evaluate new resistance genes (natural and transgenic), and all of the above in combination for a good IPM program.

Dr. Tim Dennehy (entomology), University of Arizona, provided comments about resistance management of pink bollworm and deployment of Bt cotton in Arizona including: 1) baseline susceptibility evaluations for pink bollworm (*Pectinophora gossypiella* Saunders, PBW) populations in Arizona, 2) impact of Bt cotton on biological control, and 3) refuge strategy development, validation, and implementation.

Dr. D. D. Hardee (research cotton entomologist), USDA-ARS at Stoneville, MS commented on the 1996 and 1997 resistance monitoring strategy work for cotton bollworm and tobacco budworm in Bt cotton in 4 cotton-growing states. Preliminary efforts have shown that there were no shifts in the baseline susceptibility to CryI(A)c for either cotton bollworm (*Helicoverpa zea* Boddie, CBW) or tobacco budworm (*Heliothis virescens* Fabricius, TBW).

### **C. The use of "Public Good" as a criterion for the requirement of resistance management plans**

The Agency sought comment on whether “public good” should be used as a criterion triggering the requirement of resistance management. EPA also sought comment on how “public good” should be defined. The “public good” criterion was first proposed by the Pesticide Program Dialogue Committee (PPDC) in July 1996. However, the PPDC were unable to define what constituted a “public good” except that they agreed that Bt microbial and Bt plant-pesticides were in the “public good” and should be protected. The PPDC stated that EPA was correct to require resistance management plans as part of the registration for Bt plant-pesticides.

Most individuals did not specifically address the issue. Commenters from industry indicated, in general, that the “public good” criterion was not a useful criterion as a basis for requiring resistance management plans as a condition of registration. Pioneer Hybrid International’s comments are particularly illustrative of industry’s viewpoint, they indicated that “public good” was an ill-defined concept, not based in FIFRA, and singles out Bt plant-pesticides for additional requirements. Comments from all stakeholders touched on the “societal” nature of this question. There were comments from academia and industry on the fact that FIFRA requires the Agency to make a risk/benefit decision to register a pesticide and, therefore, if a pesticide is registered, it is

in the “public good”. Some felt that mandating resistance management plans only for Bt plant-pesticides and not other pesticides is, as some researchers from Texas A & M noted, “scientifically and logically flawed.”

Dr. Kimberly Stoner (entomologist), Connecticut Agricultural Experiment Station in New Haven stated that “any pesticide discovered and developed with public funds or even grants from the state or federal governments should be considered a public good.” Dr. John Van Duyn (entomologist), North Carolina State University wrote that “this concept is potentially so encompassing that it will cause fear in most Americans, from the common and deeply rooted suspicion in the government.”

However, Dr. Mark Whalon from Michigan State University and a set of Texas A & M researchers provided an extensive evaluation of “public good.” The Texas A & M group outlined three major criteria to determine public good: 1) Impact on product output, costs of production and product price. 2) Impacts on the level of pesticide use weighted by the potential positive and adverse impacts on health and the environment. 3) Impacts on the research agenda and the level of support/effort. Public good would be defined by these criteria which consider the short-run and long-run effects expressed on a present value basis of the various impact groups.

Dr. Mark Whalon stated that “susceptibility genes in pests are natural resources” which are in the public good and should be protected. He comments that “the loss of susceptibility genes through the overuse of pesticides constitutes a tragedy of the commons no less significant than polluted air, water or contaminated food. All mankind suffers from the consequences of this genetic over-exploitation which can be and should be prevented. It is particularly tragic in that resistance can be delayed even ameliorated indefinitely with proper EPA-mandated Resistance Management Plans (RMP) which could be required as EPA has already done for some Bt-transgenic plants.” Dr. Whalon believes that RMPs should be in place at the time a new pesticide is introduced into the market, particularly where the target is a pest that has already exhibited a history of resistance. He states that RMPs should be based on five principles: 1) diversify mortality mechanisms in pest populations (IPM), 2) manage susceptible genes by providing refuge for untreated pests to survive or manage immigration of susceptible insects in treated populations, 3) establish base-line susceptibility of target pest populations and, where possible, monitor these populations as a regular course of operations, 4) when resistance develops, investigate the mechanisms and inheritance pattern to aid the development of better RMPs in other areas, 5) develop communication and education programs that aid the introduction, adoption, and maintenance of RMPs.

The Union of Concerned Scientists indicated that “Bt is a public good that should not be squandered.” This viewpoint was echoed by comments from private citizens, organic farmers and grower groups, and public interest groups.

#### **D. Performance of Bt cotton**

EPA sought comment on the performance of Bt cotton in the field. The Agency sought information regarding reported control failures for Bt cotton in 1996, possible evaluation tools concerning these failures, and implications on future resistance management efforts. Comments are summarized below. Discussion of the resistance management impacts of cotton bollworm control in Bt cotton fields will be discussed in the section IV below.

Comments received from private citizens, organic farmers and grower organizations, and public-interest groups stated a belief that the inability of Bt cotton to control cotton bollworm during the 1996 growing season showed that the “high dose strategy” was flawed. Therefore, they felt that the Bt cotton resistance management plan should be reevaluated before the 1997 growing season. Most urged EPA to suspend the registrations of all Bt plant-pesticides and to hold a FIFRA Science Advisory Panel (SAP) meeting to reevaluate the resistance management plans for Bt cotton and Bt corn.

Comments received from Monsanto, the National Cotton Council, academic/USDA scientists, and cotton farmers indicated that Bt cotton performance in 1996 was excellent. These comments stated that there was no breakdown in the Bt gene technology. Individuals indicated that there was an unusually high infestation of cotton bollworm in the Cotton belt (south Texas, mid-south and southeastern growing regions). Some of these infestations on Bt cotton required supplemental insecticide treatment.

Monsanto and a number of entomologists noted that cotton bollworm is not as sensitive to the Bt toxin as tobacco budworm and this information was published in the scientific literature before commercialization of Bt cotton (Bradley, 1995; Mahaffey *et al.*, 1994). Dr. Van Duyn, an entomologist from North Carolina State University, stated that his research showed the lack of very high efficacy in Bt cotton for cotton bollworm and that Bt cotton was not a stand-alone technology when high populations of cotton bollworm were encountered.

Monsanto commented that they undertook a number of studies following reports of “Bt cotton failure in 1996” and tested for cotton bollworm susceptibility and Bt expression in Bt cotton areas affected by high cotton bollworm infestations. They found no change in cotton bollworm susceptibility to the CryI(A)c delta endotoxin and in Bt expression levels in the plants as compared to the baseline susceptibility levels for these locations. These studies showed no detectable level of resistance in these populations. Growers realized a \$34/acre benefit growing Bt cotton versus non-Bt cotton.

Academics and the National Cotton Council all noted that there could be improvements in communication on cotton bollworm control in Bt cotton with the public, growers, and consultants. The National Cotton Council indicated that scouting practices had previously focused on the top six inches of the plant. As a result of the 1996 Bt cotton growing season, modified scouting practices in Bt cotton will now be needed to examine further down in the plant canopy especially during peak bloom periods (cotton bollworm eggs were found on flowers or blooms on Bt cotton plants), the most critical time for cotton bollworm control in Bt cotton. Dr.



Blake Layton, extension entomologist from Mississippi pointed out that producers and consultants in Mississippi were cautioned via the 1997 Cotton Insect Control Guide, the weekly Cotton Insect Situation Newsletter, the Cotton Insect Telephone Hotline, and Extension Publication 2108, “Insect Scouting and Management in Bt-transgenic Cotton” that Bt cotton may require treatment in cases where high populations of cotton bollworm occur.

Two cotton farmers from Alabama and Mississippi, both of whom planted Bt cotton in 1996, indicated that Bt cotton had excellent control of tobacco budworm and significantly reduced cotton bollworm populations as well. The Mississippi farmer noted that 1300 growers on nearly 450,000 acres planted Bt cotton in 1996. This grower indicated that less than 10% of his acres were treated for cotton bollworm. The Alabama farmer noted that 77% of all of the cotton acres planted in Alabama were Bt cotton. He did not spray one acre of his 6000 acre farm for cotton bollworms. The National Cotton Council and Dr. Blake Layton (extension entomologist from Mississippi State University) pointed out that applications for cotton bollworm control went from 3.3 applications for non-Bt cotton to 0.3 for Bt cotton in Mississippi. This type of reduction in insecticide applications for cotton bollworm control was also noted for other states employing Bt cotton.

Several entomologists discussed the subject of effective refuge size in their written comments. These experts commented that the current refuge options may not be large enough to produce a relatively high number of susceptible cotton bollworms to mate with any resistant insects that may develop on Bt cotton with only a moderate dose for cotton bollworm. The presence of a refuge can still mitigate the effects of resistance development even in the absence of a high dose. Dr. Mike Caprio from Mississippi State University commented that “while it is true that a high dose makes the refuge strategy much more effective, we have shown in simulations of foliar applications of Bt that even survivorship rates as high as 20% could still delay resistance 5-fold compared to the rate of resistance evaluation in the absence of the refuges.” He encouraged the pyramiding of multiple genes in cotton if such genes increase mortality of cotton bollworm to delay resistance development in this pest. Dr. Fred Gould (entomologist), North Carolina State University, indicated in his written comments that there wasn’t a high dose strategy for cotton bollworm and pink bollworm, but rather a moderate dose approach for cotton bollworm and that the actual effective refuge size should be greater than the current 4% in Bt cotton. Gould recommended that the effective refuge size for Bt cotton should be increased to be at least 30% non-Bt cotton. Texas A & M entomologists commented that if the acreage of alternate hosts such as corn, sorghum and soybean is greater than the Bt cotton acreage, then a 20% or greater sprayed non-Bt cotton refuge may be adequate for managing resistance to tobacco budworm and cotton bollworm. They recommend that of the two EPA mandated refuge options for Bt cotton, the 4% non-sprayed refuge option should be replaced with just the 20% sprayed refuge option. Comments from the National Cotton Council and University of Arizona indicate that pink bollworm control was excellent.

## **II. Bt potato resistance management**

This section will discuss resistance management activities and results for 1996 and discuss any lessons learned regarding resistance management since Cry IIIA delta endotoxin was registered in potatoes in 1995. Based on EPA's analysis of the registrant's voluntary resistance management strategy and comments received from the Science Advisory Panel Subpanel on plant-pesticides that the registrant's voluntary resistance management strategy was scientifically adequate (March 1, 1995, see OPP docket, OPP-00401), no requirements related to resistance management were imposed on the product registration of CryIIIA delta endotoxin in potatoes. Voluntary interaction between the registrant and EPA was recommended and certain areas of research and monitoring were suggested. However, Monsanto/Naturemark requires a mandatory refuge for each of its growers to follow and the overall Bt potato resistance management strategy is being refined as more data become available. An annual report on the status of resistance management activities was not required as part of the registration agreement, but a 1996-97 status report of resistance management activities was provided to EPA voluntarily by Monsanto/NatureMark on July 2, 1997. Material provided during the public hearings, scientific publications, personal communications, EPA's Fact Sheet (U.S. EPA, 1995a), Agency Reviews (December 23, 1994 and May 2, 1995 regarding pesticide resistance management) and Monsanto/NatureMark's 1996-97 status report (July 2, 1997) will be included in this analysis.

### *Background*

The Agency granted a conditional registration without an expiration date for the Cry IIIA delta endotoxin from *Bacillus thuringiensis* subspecies *tenebrionis* in potato to control Colorado potato beetle (*Leptinotarsa decemlineata* Say, CPB) in May 1995. This was the first registration of a plant-pesticide. Bt microbial pesticides with specific activity for CPB have been registered for a number of years (e.g. M-trak, Foil, Novodor, Raven). The major difference between the Bt microbial pesticides and the CryIIIA delta endotoxin genetically-engineered into potatoes (Bt potato) is the level of control throughout the growing season. Bt microbial insecticides have a short persistence and are only effective against young larvae, necessitating exact timing and several applications to achieve control. The expression of the CryIIIA delta endotoxin in the leaves of Bt potato is at a high enough level to be effective against all stages of the beetle and protection is sustained throughout the season.

Both the Agency and the Science Advisory Panel Subpanel on plant-pesticides (meeting held March 1, 1995, see OPP docket, OPP-00401) reviewed Monsanto's resistance management plan for Bt potato. Review of the resistance management plan was part of the Agency's risk/benefit decision-making process for registering the Cry IIIA delta endotoxin in potato. The Agency and the SAP determined that the resistance management plan for Bt potato was scientifically sound and workable. The SAP stated that the resistance management plan is a "scientifically credible Colorado potato beetle resistance management protocol." For the Bt potato, the SAP recommended that Monsanto provide the Agency with a specific resistance monitoring plan and requested that specific recommendations be developed on what course of action should be taken if resistance should be discovered. It was the opinion of the SAP that Monsanto should work with EPA on developing a long-term resistance management plan, but that such plans should not be a

formal condition of registration. EPA agreed with this assessment for Bt potato as the pesticide was only for the control of the CPB, the CryIII $\delta$ A delta endotoxin was at a high dose, and existing Bt microbial products had only limited residual activity and only worked for early instars of this pest. In addition, the CPB has a limited host range and limited mobility. EPA recommended further information be collected on reproductive strategies for CPB with respect to gene flow, optimization of refuge strategies, continued development of monitoring plans, development of a discriminating dose assay, continued development of grower education materials, continued refinement of IPM recommendations, and continued development of novel CPB control mechanisms involving different modes of action.

The SAP further agreed with the seven elements, described by OPP, that need to be addressed to develop an adequate resistance management plan for Bt plant-pesticides. These elements are: (1) Knowledge of pest biology and ecology, (2) Appropriate gene deployment strategy, (3) Appropriate refuge, (4) Monitoring and reporting of incidents of pesticide resistance development, (5) Employment of IPM, (6) Communication and educational strategies for the use of the product, and (7) Development of alternative modes of action.

#### *Bt potato acreage in 1996 and 1997*

Although no specific reporting requirements were required as part of the registration in 1995, EPA recommended the continued development of a data base to monitor the use of the genetically modified potatoes and correlate possible resistant reports with the use sites. Based on three-year averages, about 1 million acres of fall potatoes are planted in the U.S. annually. According to information provided in the 1996-97 status report, about 10,000 A (or 1% of the total) in 1996 and 25,000 A (or 2.5% of the total) in 1997 were planted in Bt potatoes (marketed as NewLeaf® Russet Burbank and NewLeaf® Superior and NewLeaf® Atlantic varieties) in the U.S. Acreage information was gathered from 94 of 112 total customers in 1996. The proportion of NewLeaf® potatoes on these farms ranged from 0.1% to 69% of total potato acreage. Farm size ranged from less than 500 A to 5000 A.

#### *Analysis of Resistance Management Strategy*

##### *High Dose is Adequate*

All available evidence supports the Agency's original finding (December 23, 1994 and May 2, 1995) that NewLeaf® potatoes constitutively express the CryIII $\delta$ A delta endotoxin at sufficiently high doses to kill all susceptible individuals including resistant heterozygotes. NewLeaf® potato hybrids are maintaining a "high dose" expression of CryIII $\delta$ A throughout the plant to provide a "high dose strategy" for resistance management. This conclusion was supported by the March 1, 1995 SAP (published report, see OPP docket, OPP-00401) and public comments from entomologists. According to NatureMark's 1996-97 status report, the levels of CryIII $\delta$ A delta endotoxin expression appear to be "approximately 10-50 times higher than the LC<sub>99</sub> for CPB larvae."

### *CPB resistance to Bt Endotoxins*

EPA did not require as part of the registration more information on the biology of CPB resistance and the potential for cross-resistance. However, EPA and the SAP recommended that this information would be useful to further refine the long-term resistance management strategy. Field resistance to microbial Bt or to Bt potatoes expressing the CryIII $\delta$ A delta endotoxin has not been reported to date. However beginning in 1987, Whalon's group selected field and laboratory colonies with M-One, a commercial foliar Bt spray containing the CryIII $\delta$ A delta endotoxin, to study the selection and the inheritance of resistance (Whalon *et al.*, 1993). Whalon's group developed resistant strains that were 59 times more resistant than the unselected strain. No cross-resistance was observed between organophosphate, carbamate or synthetic pyrethroid resistance and the CryIII $\delta$ A-resistant strain. However, this Bt resistant strain feeding on transgenic potato petioles producing the CryIII $\delta$ A delta endotoxin showed stage-specific mortality (Wierenga *et al.*, 1996). Results from this study indicated that the third instars were typically less sensitive than the first and second instars to the Bt delta endotoxin. The older instars had a significantly slower rate of development than those feeding on conventional non-Bt foliage. Resistant second instar larvae experienced less than 50% mortality after 96 h of feeding on a Bt potato plant. After two weeks of feeding on a Bt potato plant, resistant adult beetles experienced only a 25% mortality in comparison to susceptible adults which did not survive.

These studies provide important information about the limitation of a seed-mix refuge strategy for CPB. Larger larvae surviving on non-Bt plants could move onto Bt plants and receive a sublethal dose of the CryIII $\delta$ A delta endotoxin and thus speed up the selection process for resistance. Modeling studies performed by Mallet and Porter (1992) indicate that seed mixes could enhance selection if there was significant larval movement within a field. Further selection studies examined the inheritance of resistance. Research by Rahardja and Whalon (1995) indicated that the genetic inheritance of CPB resistance to the Bt CryIII $\delta$ A delta-endotoxin was conferred by incomplete dominance genes. After 35 generations of intense selection to the CryIII $\delta$ A delta endotoxin, the resistance ratio was >700-fold for Bt-resistant CPB compared to the susceptible strain and resistance development resulted in significant fitness costs, i.e., prolonged larval development, reduced larval weight, shortened oviposition period, reduced egg-mass size, and reduced fecundity (Trisyono and Whalon, 1997). Further experiments indicated that these Bt-resistant CPB could survive on transgenic Bt potato plants expressing the CryIII $\delta$ A protein for a short period of time, although none of the eggs produced by these adults were viable (DiCosty and Whalon, 1997). Collectively, these studies provide information about the nature of CPB resistance under intensive-selection to the CryIII $\delta$ A delta endotoxin in the laboratory and suggest that incorporation of an effective structure refuge into a resistance management strategy is desirable to slow the potential for resistance to develop. Further field research is needed to validate the resistant management models, to study the CPB adult and larval movement, mating behavior, and to study reproductive effects of CPB feeding on Bt potatoes.

### *Refuge*

All available evidence continues to support EPA's original conclusion that a "structured" refuge is necessary and that the success of the high dose strategy will be compromised if there is no effective refuge. Seed mixes were eliminated as a realistic alternative for CPB resistance management because of the potential for sublethal exposure to the CryIIIA delta endotoxin by later instar larvae surviving on a non-Bt plant and then moving to a Bt plant. However, except for the elimination of a refuge based on a seed mix, neither the Agency nor the SAP recommended a specific "structured" refuge arrangement and relative size of Bt potato and refuge plots prior to registration of CryIIIA delta endotoxin in potatoes in May 1995. That is, EPA did not include a refuge requirement as a part of the registration.

Prior to 1997, the refuge concept was included in Monsanto/NatureMark's resistance management plan as a recommendation rather than a requirement as part of the Technology Agreement. Growers were instructed by NatureMark to maintain at least 20% of farm potato acres as non-Bt expressing potatoes that could be treated with conventional insecticides for CPB. As noted above, compliance with this recommendation was high. The proportion of NewLeaf® on these farms ranges from 0.1% to 69% of total potato acreage of 94/112 growers surveyed by NatureMark.

Beginning in the 1997 growing season, commercial growers were required by NatureMark's Technology Agreement to maintain at least 20% of their potato acreage as non-Bt expressing varieties. The recommended spatial arrangement and treatment of these refuge acres is noted in NatureMark's Resistance Management Guide. Refuge acres should be growing in close proximity to NewLeaf® fields. Refuge acres may be treated for CPB, but treatments should be with foliar (non-Bt insecticides), rather than systemic insecticides to allow enough susceptible insects to survive. It is recommended that NewLeaf® potatoes should be vine-killed prior to non Bt-expressing potatoes. If there are resistant insects surviving on NewLeaf® hybrids, they should move to the non-Bt expressing potatoes and mate with susceptible CPB. NatureMark reports that compliance with this refuge requirement and other requirements of the Technology Agreement will be monitored.

A refuge requirement is recommended, but not required by NatureMark for seed growers because they maintain numerous varieties on their farms. As a result, there is an ample supply of non-Bt expressing refuge as a natural component of seed production. Historically, CPB resistance has not been a problem in seed producing areas.

#### *Surveillance and Tracking (Monitoring)*

In 1995, the SAP and EPA recommended that a detailed monitoring program and a remedial action plan should be developed and instituted. The monitoring plan should include sampling sites, timetable for development, education of growers on sampling for resistance, collecting specimens to evaluate for resistance, and providing specific recommendations on how to eradicate resistant individuals to prevent survival of a resistant population. EPA also recommended the development of a discriminating dose assay. NatureMark has provided a summary of the baseline

susceptibility work, development of a discriminating dose assay, and a detailed monitoring/surveillance program description including an appropriate remedial program. NatureMark has a 1-800 number for growers to report unusual CPB survival or for other technical information.

NatureMark reports that the baseline susceptibility work for CPB populations to the CryIII<sup>A</sup> protein has been completed. During a four-year period beginning in 1992, a total of 79 geographically distinct populations were collected from commercial potato farms in 15 states and two provinces of Canada and were assayed for susceptibility (work completed by Dr. Galen Dively, Dept. Of Entomology, University of Maryland). Results from this study indicated a seven-fold difference in CPB baseline susceptibility to the CryIII<sup>A</sup> protein between the various populations. A discriminating concentration of 25 µg protein/ml of diet was selected to test for shifts in susceptibility in suspect CPB populations that may be identified in the future. This concentration killed 26% of the Whalon resistant strain (Whalon *et al.*, 1993) compared to 100% of the standard New Jersey susceptible population.

NatureMark has developed user guidelines explaining the deployment of refuges and monitoring requirements and has put into place an outreach program in cooperation with seed suppliers and extension entomologists to look for unexpected levels of CPB survival. NatureMark has developed a rapid serological test that can be used to identify plants containing the Bt protein in one hour. If plants are confirmed to be NewLeaf, then “suspect” larvae will be shipped to the University of Maryland for bioassay to determine actual susceptibility to Bt. The University of Maryland has a two level testing program that would be implemented if there is a “suspect” CPB population. This program is designed to detect localized shifts in susceptibility level rather than shifts in resistance gene frequency. Monitoring shifts in resistance gene frequency would be cost-prohibitive. Level 1 testing will have the University of Maryland bioassay laboratory expose a large number of first instars to the discriminating concentration and determine if the mortality response is statistically different from the baseline response. The level 1 testing will take approximately one week. The level 2 testing will further quantify the concentration-mortality response and provide information on follow-up actions such as intensified field surveillance in and around the collection site or fact-gathering to assess reasons for the unusual response. If CPB are found to be resistant to Bt, they can be treated immediately with a conventional insecticide to prevent further reproduction and movement.

NatureMark reports in its 1996-1997 status report of resistant management activities that there were two situations in which growers alerted NatureMark of CPB larvae surviving on NewLeaf® plants. In both cases, the plantings were non Bt-expressing plants rather than NewLeaf® plants due to planting errors. NatureMark indicates that they confirmed that the plants did not contain the Bt gene.

### *Communication and Training*

NatureMark mandates certain activities under its Technology Agreements with growers and

charges a \$32 per acre technology usage fee in addition to the cost of the seed potatoes. NatureMark contacted each NewLeaf® grower personally by telephone and visited each farm to discuss resistance management and IPM. This effort helped to ensure that all enlisted growers had all of the technical information needed to use the product appropriately and successfully.

Monsanto/NatureMark has consulted with research and extension entomologists in the development of its resistance management program. NatureMark has prepared a simple one-page summary of its Resistance Management Guide for growers. Additional documents describe regional pest management recommendations, including non-target pest scouting and choices for economical and selective pesticides to use in combination with NewLeaf® potatoes. Each grower receives all of the technical information including resistance management requirements and recommendations prior to signing NewLeaf®'s Technology Agreement and again at the completion of signing. These materials are available at trade shows, grower meetings, and through direct mailings. NatureMark has developed an alliance with three major chemical distributors, Wilbur Ellis Co., United Agri Products, and Simplot Soilbuilders to provide crop service and support to NewLeaf® growers. Field representatives from these companies are trained on NewLeaf® Best Management Practices including insect resistance management. These "Service Partners" are another mechanism to ensure compliance with the resistance management plan.

#### *Public comments*

Of the 100 comments received as a result of the two public hearings held on Bt plant-pesticide resistance management this year, only four were specifically related in some fashion to Bt potato resistance management strategies.

Dr. David Ferro (entomologist), University of Massachusetts and a member of the SAP Subpanel in 1995 on plant-pesticides who originally reviewed Monsanto's/NatureMark's resistance management plan, commented that NatureMark should modify instructions on how the 20% non-Bt expressing acreage should be managed. "The 20% refuge may be too far away from the NewLeaf® plantings to insure mating between RR and SS individuals." He recommends that no more than 80% of the area in any single field be planted to a NewLeaf® cultivar and that growers must manage the 20% refuge using IPM guidelines. He also expresses some concern that the use of foliar and soil-applied imidacloprid, if not managed properly, might eliminate susceptible insects in the refuge.

Praxis, an integrated biological cybernetics company based in southwest Michigan, commented that CPB resistance management plan for Cry IIIA-expressing potatoes is flawed because of the potential impact on beneficial insect populations. However, written comments provided by the Wisconsin Potato and Vegetable Growers Association and the Department of Entomology, University of Wisconsin-Madison, states that populations of beneficial insects are significantly higher in Bt potatoes than in those managed with conventional insecticides where "reductions of 50-75% are common." In 1996 field experiments, beneficial arthropod populations were higher

than those treated with any other insecticides. However, the predator populations were not high enough to control late-season aphid populations. Their comments support NatureMark's 1997 resistance management guidelines: 20% non-Bt refuge, use of alternative controls, and a monitoring program for Bt susceptibility.

The Canadian Pest Management Regulatory Agency provided a copy of the report of the October 1996 meeting on integrated management of CPB. This report, while good, does not include a discussion of Bt potato expressing the CryIII $\delta$  endotoxin.

### *Summary*

NewLeaf potato hybrids are maintaining a "high dose" expression of CryIII $\delta$  throughout the plant to provide a "high dose strategy" for resistance management. NatureMark, through its 1997 Technology Agreement with NewLeaf® growers, mandates a 20% non-Bt expressing refugia. It appears that compliance with the conditions in the Technology Agreement was excellent. NatureMark has developed a discriminating dose assay, a surveillance and remedial action plan, and an extensive grower education communication and training program to convey appropriate resistance management tactics. IPM and scouting are discussed in the technical material provided by NatureMark. NatureMark has adopted all of EPA's recommendations regarding resistance management although no specific requirements were mandated as conditions of registration. Evidence suggests that the resistance management strategy adopted by NatureMark is a good one, although there is some concern over treatment of the 20% non-Bt expressing refuge and level of beneficial insects in the field. Adoption of NewLeaf® technology may be slowed by the rapid acceptance and implementation of imidacloprid insecticide as a new chemical control of CPB.

### **III. Bt corn resistance management**

This section will discuss resistance management activities and results for 1996 and discuss any lessons learned regarding resistance management since the first CryI(A) $\delta$  endotoxin was registered in corn in 1995.

The following materials were used in preparing this section of the paper: material provided during the public hearings, scientific publications, EPA's Fact Sheets (U.S. EPA 1995b; U.S. EPA, 1996a, b; U.S. EPA, 1997), Agency Reviews regarding pesticide resistance management, Research and Extension Entomologists of the USDA North Central Regional Research Project (NC-205), Ecology and Management of European Corn Borer and Other Stalk-Boring Lepidoptera, North Central Regional Extension Publication, NCR 602 entitled "Bt-corn & European Corn Borer - Long-Term Success Through Resistance Management" (Ostlie *et al.*, 1997), Ciba Seeds/Novartis Seeds and Mycogen Plant Sciences 1996-97 status reports, Letters from Ciba Seeds/Novartis Seeds (November 20, 1996) and Mycogen (December 9, 1996) regarding silk and kernel expression in Event 176 hybrids, Research Data on Corn Earworm Relative to Resistance Development and Monsanto's Plans for Producing Resistance Predictive



Models (MRID 442094-01), and a discussion of 1997 research plans.

### *Background*

The Agency granted the first two conditional registrations of the *Bacillus thuringiensis* subspecies *kurstaki* CryI(A)b delta endotoxin and the genetic material necessary for its production in field corn to control European corn borer (*Ostrinia nubilalis* Huebner, ECB) in May 1995 to Ciba Seeds (now part of Novartis) and Mycogen Plant Sciences. The first commercial plantings of Event 176 hybrid Bt corn were in 1996. At the time this paper was prepared, the EPA had registered five Bt plant-pesticides for commercial use in field corn: 176 (Ciba/Novartis Seeds and Mycogen Corp.), BT11 (Northrup-King/Novartis Seeds), MON801<sup>1</sup> and MON810 (Monsanto) and DBT418 (DEKALB Genetics Corp.). Each of these transformation events is trademarked and various seed companies license each event. Understanding these events and how they effect performance is crucial to the wise selection of corn hybrids and to appropriate resistance management.

Resistance management strategies for the *Bacillus thuringiensis* subspecies *kurstaki* CryI(A)b or CryI(A)c delta endotoxin and the genetic material necessary for its production in field corn have been reviewed by the Agency. Summaries of these analyses and the terms and conditions of the registration including the resistance management requirements required are provided in the FACT sheets for the individual registrations (U.S. EPA, 1995b; U.S. EPA, 1996a, b; U.S. EPA, 1997). Each of these conditional registrations will automatically expire on midnight April 1, 2001. EPA will reevaluate the effectiveness of each registrant's resistance management plan before April 1, 2001 to decide whether to convert the registration to a registration without an expiration date. Experimental Use Permits (EUPs) have been granted and amendments to registrations are pending for use of a CryI(A)b delta endotoxin in sweet corn (Rogers Seeds/Novartis Seeds) and in popcorn (Novartis Seeds). An application is pending for the registration for a Cry 9(c) delta endotoxin in field corn (Plant Genetic Systems/AgrEvo).

At the time of registration, there were some scientific questions related to long-term resistance management of the CryI(A)b and CryI(A)c delta endotoxins expressed in corn. Because of these questions, the Agency imposed specific resistance management requirements. EPA required as part of the registration, development of a monitoring program (surveillance and remediation), grower education, and maintenance of a confidential sales database. Each registrant is required to submit annual progress reports on results and conclusions from research (including scientific literature) as they become available in the following areas: 1) information on ECB pest biology and behavior, 2) feasibility of refuge options, 3) development of discriminating dose concentration assay, 4) effect of corn producing the CryI(A)b or CryI(A)c delta endotoxin on pests other than ECB including CEW, and 5) the biology of ECB resistance and cross-resistance. Both Novartis Seeds (Ciba Seeds) and Mycogen Plant Sciences have submitted their 1996 progress reports (no

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<sup>1</sup>MON 810 is the major commercialized event and is the only event discussed in detail in this paper. MON 801 was registered, but not commercialized.

other delta endotoxins were registered in corn for full-scale commercial release prior to the 1996 growing season). Progress reports for 1997 for all Bt plant-pesticides registered for use in corn will be submitted to EPA by January 31, 1998.

It is recognized that structured refugia coupled to a high dose expression strategy are two of the key resistance management factors for managing pest resistance to Cry delta endotoxins in corn. However, successful grower education and training are essential to implementation of any resistance management plan. In addition, it is recognized that long-term resistance management will involve other IPM practices in addition to the use of Bt corn. In all cases, all of the general elements of a resistance management plan were addressed by the registrants and reviewed by EPA. These elements include pest biology, Bt dose deployment, refugia, monitoring for ECB resistance, susceptible nontarget lepidopteran pests, potential for cross-resistance development, integrated into an IPM program, grower education and communication, remedial action plan, and development of alternative pesticides with different modes of action.

There are two major resistance concerns: 1) development of resistance in the primary target pest, ECB and 2) development of resistance in secondary target pest resistance in corn earworm (CEW) [also known as the cotton bollworm, sorghum headworm, tomato fruitworm, and soybean pod borer (*Helicoverpa zea* (Boddie))]. EPA concluded that to manage resistance effectively and to develop an effective, long-term resistance management strategy, specific data needs (including target and secondary pest biology and ecology, population dynamics (modeling), refugia, cross-resistance, baseline susceptibility and discriminating dose determination), mitigation measures (remedial strategies and limitation of Bt corn acreage in the South for Bt corn hybrids producing Bt protein in the silks and kernels), reporting, and monitoring were required as part of the registration. No specific structured refuge requirements were mandated at the time of registration, but the registrants were required to collect research data regarding different refuge strategies in order to determine the necessary arrangement and relative size of Bt and refuge plots for a long-term resistance management plan. However, seed mixes were eliminated as an effective refuge strategy because of relatively high ECB larval movement. Because of this movement, there is a threat that ECB larvae would be exposed to sublethal doses of the Bt toxin in Bt plants and then move to non-Bt plants allowing selection for resistance to occur. By August 9, 1998, a draft refuge strategy must be submitted to the Agency and a final refuge strategy submitted by January 31, 1999. The registrant must implement an EPA approved “structured” refuge plan or an EPA approved alternative resistance management plan no later than April 1, 2001. Registrants are required to discuss the development and implementation of the refuge plan and alternative resistance management practices with EPA throughout the development and the implementation.

Monsanto Co. and Dekalb Genetics mandated certain activities under their Technology Agreements with growers and charged a \$32 per acre technology usage fee in addition to the cost of the seed. The Technology Agreements requires that growers who use MON810- or DBT418-derived hybrids must implement either a 5% unsprayed non-Bt refuge or a 20% sprayed non-Bt refuge. Novartis Seeds and Mycogen Seeds do not require any specific refuge option to be

implemented, but do recommend the use of a refuge.

EPA, a number of entomologists, environmental groups, and other stakeholders have expressed concern regarding the impact of CryI(A)b- and CryI(A)c- expressing corn on CEW in those areas where CryI(A)c-expressing cotton is grown. EPA imposed restrictions on the number of acres allowed in the South on Bt corn hybrids expressing the Bt delta endotoxin in silks and kernels, at present this would include events MON810-, BT11-, DBT418-derived hybrids, but not Event 176-derived hybrids. A total of 200,000 A was allowed in the South: 100,000 A each for MON810- and BT11-derived hybrids and none for DBT418-derived hybrids. Dekalb's Bt plant-pesticide expressing the CryI(A)c delta endotoxin, DBT418, was not registered for use in parts of the South because the Agency determined that the resistance risk was too high to allow additional Bt corn plant-pesticides that express Cry delta endotoxins in silk and kernels to be used there. Based on receptor binding experiments, CryIA(b) or CryIA(c) delta endotoxins have similar binding characteristics, indicating that there might be a high degree of cross-resistance to these two toxins. In addition, the CryI(A)c delta endotoxin is also registered in cotton increasing the potential for Bt resistance in pests exposed to this toxin in both corn and cotton. Event 176 CryI(A)b-expressing corn was not restricted in the South because it has only trace (<8 ppb) levels of the delta endotoxin in silks and kernels and is not expected to select for second generation CEW resistance. In addition to sales restrictions, research data and model development were required on all the Bt hybrids registered to evaluate the potential impact of Bt corn on Bt resistance management programs in areas growing corn and cotton.

Silk and kernel expression in Bt corn hybrids will likely increase the selection for CEW resistance especially in cotton-growing areas. If there is silk expression of the CryI(A)b/CryI(A)c delta endotoxin at sufficient levels to select for resistant CEW, then resistant CEW could move from Bt corn to cotton/Bt cotton posing potentially significant problems in cotton or Bt cotton or potentially in other crops affected by CEW. Where corn and cotton acres are in close proximity, there will be migration of second generation CEW from silk-stage Bt corn to cotton (including Bt cotton) and other crops. In the southeastern U.S., virtually all second generation CEW funnel through corn where they complete development on the ear of this preferred host. Selection for CEW/CBW resistance could be accelerated if Bt corn hybrids became widely adopted in the South if adequate resistance management was not adopted. In the South, there are 3 to 6 CEW generations and in the North, there are 1 to 2 generations. Thus, CEW in the South are potentially subject to higher levels of exposure to the Bt delta endotoxin than CEW in the North. CEW only overwinter in the South. However, the development of CEW resistance to Bt in the North is also a concern. The major source of CEW in the northern corn belt is adults flying or being carried on the prevailing winds from the southern states each year. Should CEW resistance to Bt toxins develop in the South, it could be equally damaging in the northern states growing Bt corn each season. In the South, there would be a higher selection pressure in areas in which Bt corn and Bt cotton are in close proximity and in areas in which Bt microbial pesticide products are used. Resistant CEW could lead to the failure of Bt microbial pesticides used on cotton and other crops or to the failure of Bt cotton and Bt corn, and other crops both in the South and in the North for control of CEW. Although the risk of loss of Bt and increased use of chemical

insecticides cannot be quantified, EPA believes this risk is real. There could also be negative impacts on organic farmers from the loss of Bt. EPA addressed these risk in issuing the Bt corn registrations.

While the theory of high dose expression coupled to effective structured refugia is relatively straightforward, its implementation and enforcement have been controversial. Long-term Bt corn resistance management is complicated by the following circumstances: (1) there are multiple competitors for the technology (there is only a single registrant involved in both Bt potato and Bt cotton), (2) there are different Bt delta endotoxins and transformation events being put forward by the various companies with differences in levels of expression through the plant, (3) research efforts are not consolidated, and (4) there are a large number of states, large number of growers, and tremendous corn acreage throughout the U.S. involved. With all of these factors in mind, there is disagreement among stakeholders as to what is (1) the appropriate arrangement and relative size of Bt corn and refuge plots, (2) the nature and objective of performance-monitoring activities, (3) research coordination, and (4) appropriate incentives to foster grower education and acceptance. The Agency has fostered and participated in efforts to resolve these disagreements to the satisfaction of all stakeholders and has requested public comment. One such effort to date to bring all stakeholders together has been by the USDA NC-205 group (research and extension entomologists of the North Central Regional Research Project (NC-205), "Ecology and Management of European Corn Borer and Other Stalk-Boring Lepidoptera") (Ostlie *et al.*, 1997). The NC-205 efforts will be discussed in more detail. This White Paper will analyze where EPA is in resolving these critical issues and provide recommendations on improvements for development of a long-term resistance management strategy for Bt field corn.

#### *Bt corn acreage in 1996 and 1997*

EPA required each registrant to submit to EPA annual sales data for each state indicating the number of units of Bt corn hybrids each registrant sells. Because FIFRA section 10 restricts the release of certain confidential business information, exact sales data may not be provided. However, the following information provided by the registrants is publically releasable.

Approximately 400,000 acres (about 0.5% of the total corn acreage) of Bt corn were planted in 30 states in the U.S. in 1996 and approximately 3.5 to 4 million acres (about 5% of the total corn acreage) is expected to have been planted in 1997. Based on a three-year average, there are approximately 70 to 80 million acres of corn planted annually in the U.S. If the total Bt corn acreage is in the 4 million range, then the adoption of Bt corn will have grown by about 10-fold between the 1996 and 1997 growing seasons. Part of the reason for this expected growth is the registration of new CryI(A)b or CryI(A)c delta endotoxins and the necessary material for their production (BT-11-, MON-810-, and DBT 418-derived Bt corn hybrids) in late 1996 and early 1997. Sales information for the 1997 growing season will be provided to EPA by January 31, 1998 as required by the requirements of registration.

#### *Analysis of Resistance Management Strategy*

*Expression of CryI(A)b/CryI(A)c and impact on European corn borer (Ostrinia nubilalis, ECB) - primary target pest*

The primary target of Bt plant-pesticides used in field corn is the European corn borer. All of the registered Cry toxins in corn express a dose sufficiently high to control first-generation ECB in whorl-stage corn. However, the level of control against late-season ECB generations differs between Bt plant-pesticides. That is, not all of the Bt plant-pesticides provide a “high dose” to effectively control second (or later) generation ECB. Events BT11, MON 810 and DBT418 maintain a “high dose” expression throughout the growing season in corn plants expressing the CryI(A)b or CryI(A)c for ECB control. Under heavy ECB pressure in silking corn, BT 11-, MON 810-, and DBT418-derived hybrids provide a higher level of late-season control than Event 176. Measured in terms of reduction in tunneling damage, the level of control for BT11-, MON810-, and DBT418-derived hybrids is greater than 95% for full-season control of ECB; whereas, the level of control for Event 176-derived hybrids is greater than 95% for first generation ECB and about 70-75% control of second generation ECB. There is a difference in full season ECB control when CryI(A)b or CryI(A)c is expressed only in green tissue and pollen in Event 176-derived hybrids as compared to when Cry1A(b) or Cry1A(c) is expressed in all plant tissues including silks and kernels as found in BT11-derived hybrids, and MON810- derived hybrids and DBT418-derived hybrids. Mycogen Plant Sciences, Novartis Seeds, Monsanto, and Dekalb Genetics are continuing to research the expression patterns in Event 176-, BT11-, MON810, and DBT 418-derived hybrids to determine the level of control on first and second generation ECB. These efforts will be summarized in the forthcoming registrant annual reports due to be submitted to the Agency by January 31, 1998.

*Event 176-derived hybrids - Silk and kernel expression and effect on ECB control*

Event 176 Bt corn hybrids express the Cry 1(A)b delta endotoxin in silk and kernels at less than 8 ppb and 5 ppb, respectively (Ciba Seeds/Novartis Seeds letter to EPA dated November 20, 1996). An ELISA of Mycogen Plant Science's Event 176 Bt hybrids indicated there was no detectable CryI(A)b expression in silks (Mycogen Corp. Letter to EPA dated December 9, 1996). There may be trace levels of Bt toxin in the silks and kernels, but they are below the level of detection and are not at levels thought to selection for Bt resistance by second generation CEW. Because some hatching larvae initially colonize ears to feed on silks and developing kernels, these larvae may survive on Event 176 and may tunnel later in stalks and ear shanks. The presence of second generation ECB in ears in Event 176 corn is a topic of resistance management discussions. Nonetheless, control with Event 176 corn (>95% control of first generation ECB and about 70-75% control in second generation) is better than conventional insecticide options [1996 Progress Reports from Ciba Seeds/Novartis Seeds and Mycogen Plant Sciences]. Insecticides provide 80% and 67% control of first and second generation ECB,

respectively (Ostlie *et al.*, 1997).

Field research performed by Dr. Fred Gould, North Carolina State University in 1996, indicated that ECB may be surviving on Event 176 Bt corn silks, but the results are difficult to interpret. Gould concluded that “176 line of Bt corn does not produce a high enough dose to be considered as part of a resistance management program for ECB that requires a high dose in the plant during the period when second generation larvae are present” (from Appendix VI, 1996 Growing Season Report, Ciba Seeds/Novartis Seeds, January 30, 1997).

*Expression of CryI(A)b and CryI(A)c and effects on other pests including CEW and Southwestern corn borer*

While the primary target pest for these Bt corn hybrids is ECB, the CryI(A)b and CryI(A)c delta endotoxins might suppress or control, to some degree, other lepidopteran pests. Three non-ECB pests somewhat susceptible to the CryI(A)b or CryI(A)c delta endotoxins are: southwestern corn borer, *Diatraea grandiosella* (Dyar) (SWCB); CEW; fall armyworm, *Spodoptera frugiperda* (J. E. Smith); and *Diatraea crambidoides* (Grote), southern cornstalk borer (SCSB). Selection for resistance may also occur in these other pest species susceptible to the Bt delta endotoxins and the Agency is concerned about this risk. Therefore in the conditional registrations, the Agency asked for data regarding the potential effects and the development of resistance in SWCB, CEW fall armyworm, SCSB, and other secondary pests, and took other steps to manage the risks.

Novartis Seeds and Mycogen Plant Sciences have made no control or suppression claims on their labels for the control of secondary target pests by Event 176-derived hybrids expressing CryI(A)b. Dekalb Genetics Corporation has also made no control or suppression claims on its label for the control of secondary target pests by DBT 418-derived hybrids expressing CryI(A)c. Novartis Seeds has made control or suppression claims on its label for the control of southwestern corn borer, corn earworm, and fall armyworm by BT 11-derived hybrids expressing CryI(A)b. Monsanto has made control or suppression claims for the control on its label of southwestern corn borer and corn earworm by MON 810-derived hybrids (i.e. Yieldgard™) expressing CryI(A)b. Research on the effects of CryI(A)b and CryI(A)c on other Lepidopteran pests, primarily conducted in 1996, will be discussed below.

(1) *Event 176 efficacy data on multiple secondary Lepidopteran pests (work sponsored in part by Novartis Seeds).* Pilcher *et al.* (1997) describe field and laboratory evaluations of Event 176 Bt corn on secondary Lepidopteran pests. Based on these experiments conducted in 1994 and 1995, no Bt corn effects were observed on larval survival, pupal weight, or days to adult emergence for *Agrotis ipsilon* (Hufnagel) (black cutworm) or *Papaipema nebris* (Guenee) (stalkborer); however, *Pseudaletia unipuncta* (Hawoth) (armyworm) and CEW were affected by Bt (Bt treated diets). Under field conditions,

there were no differences between Bt and non-Bt corn damage caused by black cutworm, stalkborer, CEW, and armyworm. CEW survived on and caused damage to Bt corn ears, and even though there were fewer ears damaged than the non-Bt controls, there was no difference in the number of live larvae per plant on Bt corn compared to non-Bt corn. Conversely, there were fewer feeding scars and no larval survivors on whorl-stage Event 176 corn; whereas, there were numerous feeding scars and larval survivors on non-Bt corn.

(2) *Southwestern corn borer efficacy data.* Field trials conducted at Kansas State (Bushman and Higgins) and Texas A & M (Archer) (1994-1996) [work sponsored by Novartis Seeds, Mycogen Plant Sciences, and Monsanto Co.] showed that BT11- and MON810 events provided excellent full-season control (>90%) of southwestern corn borer, while Event 176 afforded only about 20-50% control. In this situation, full-season Bt expression (constitutive expression) provided by the BT11/MON810 events was necessary to control southwestern corn borer. First brood control was excellent for all hybrids tested. The greatest difference was seen in second brood control because of the difference in expression patterns between Event 176 and BT11/MON810 hybrids. Work is continuing by Buschman and Higgins at Kansas State University to investigate the efficacy of BT11/MON 810-derived hybrids and Archer at Texas A & M to investigate the efficacy of Event 176-derived hybrids against SWCB.

(3) *Southern cornstalk borer efficacy data.* Field trials conducted at North Carolina State University by Dr. John Van Duyn in 1996 (Mycogen Plant Sciences and Novartis Seeds sponsored work) indicated that there was about 65% control of southern corn stalk borer in Event 176 Bt corn. Further efficacy testing will be done in 1997.

#### *Corn Earworm (CEW) - Effect of Bt corn on CEW survival: Field Trial Results*

Numerous field trials examined CEW susceptibility to the CryI(A)b delta toxin in Event 176 and BT11/MON810 corn hybrids. Only BT-11 and MON 810 labels make claims for control or suppression of CEW. The results of these field trials are discussed below.

#### *Monsanto-sponsored work (MON 810-derived hybrids)*

Monsanto has made control or suppression claims for the control of southwestern corn borer and CEW by MON 810-derived hybrids (i.e. Yieldgard™) expressing CryI(A)b. Monsanto was required as a condition of registration to submit available research data on CEW relative to resistance development and its plans for producing resistance predictive models by January 31, 1997. The Agency has received Monsanto's available research data on CEW relative to resistance development and plans for producing resistance predictive models (MRID 442094-01, dated January 30, 1997). This information is summarized below.

(1) CEW densities and development in MON810 were evaluated in 1995 by Dr. Fred Gould (North Carolina State University) in North Carolina. CEW growth was slowed by Bt in MON 810. This work indicates that only a third as many larvae were able to complete development in MON810 compared with the non-Bt larvae control. Dr. Gould concludes that MON810 could change the phenology of adult CEW moth flights from corn to cotton leading to a need for alteration of scouting procedures in cotton. In places and years with low CEW densities, delays in adult emergence could lead to protracted moth flights which would decrease peak earworm densities in cotton by spreading out larval hatch over time. However, protracted moth flights could also select for resistance in areas such as North Carolina where a large percentage of the CEW population is found in ear stage corn.

(2) Three field trials were conducted by Dr. Randall Higgins (Kansas St. University) in Kansas in 1996. The objective of these trials was to determine the extent in which CEW oviposition, larval densities, and larval development differ among MON810, BT11, and Event 176 corn hybrids compared to non-Bt corn hybrids. Results suggest that there are no consistent differences in CEW densities within ears between Bt and non-Bt corn hybrids. Findings also suggest that CEW development was delayed by the presence of Bt, although the magnitude of these differences varied among Bt hybrids and across time (early- vs. late-planted corn). CEW development was delayed for both MON810 and BT 11 corn hybrids in early-planted corn, but not for Event 176 hybrids. In late-planted corn, CEW development was delayed for MON810, BT11, and Event 176 corn hybrids, but was greatest for MON810 and BT11 corn hybrids.

(3) In a 1996 Kentucky field trial conducted by Dr. Ricardo Bessin (University of Kentucky), there was a reduction in CEW larval weight for all Bt hybrids tested (i.e., BT11, MON810, Event 176). The greatest reduction in weight was seen in BT11 and MON810 hybrids (82-90%) versus Event 176 (41-64%).

(4) A series of experiments was conducted by Dr. John Van Duyn (North Carolina State University) in North Carolina in 1996 to evaluate CEW whorl and ear stage infestation levels, insect development, survival, and fecundity. BT11 and MON810 corn hybrids had significantly lower levels of feeding damage from CEW compared with non-Bt controls. The number of infested ears was not consistently affected by Bt in the plant. In early-planted corn, delayed development and reduced larval weights were evident for BT11 and Event 176 corn hybrids compared with the non-Bt control (Pioneer 3394). CEW infestation in late-planted corn was more difficult to interpret because of overlapping second and third CEW generations, but larval weight reduction was observed in MON810 hybrids similar to findings in other field trials. Pupal weights, pupation time, and adult eclosion were delayed in BT11 and Event 176 corn lines compared with the non-Bt control (Pioneer 3394).

(5) In 1996 field trials conducted jointly by Dr. Galen Dively (University of Maryland) and



Dr. Ames Herbert (Virginia Polytechnic University) in Maryland, Virginia and North Carolina, on CEW survival, development and feeding injury in MON810 and non-Bt hybrids was evaluated. In addition, ovipositional rates, moth survival, and egg hatching were evaluated in the laboratory for CEW. Relative to the non-Bt hybrid, insects feeding on MON810 hybrids were delayed in development and had reduced fecundity. Evaluation of overwintering survival and construction of life tables are in progress.

*Dekalb Genetics sponsored work (DBT418-derived hybrids)*

Dekalb efficacy data indicated that the CryI(A)c protein is much less toxic to CEW than it is to ECB. Dekalb has made no label claims of control or suppression of CEW on its DBT 418 (CryI(A)c delta endotoxin and the genetic material necessary for its production in corn). Results of laboratory and field efficacy studies conducted on CEW are summarized below.

(1) The effect of the CryI(A)c protein on the growth of CEW larvae was investigated by weighing insects following exposure to a series of toxin concentrations. Results were plotted as the percentage of control insect weight vs. the concentration of CryI(A)c. CryI(A)c levels in DBT418 silk and ear leaf tissue were sufficient to slow larval growth of CEW larvae (>80%), but not adequate to kill CEW directly. CEW damage to the ear on DBT418 plants does not appear to be significantly reduced. However, it is likely the growth inhibition will result in increased CEW field mortality because of increased predation and parasitism, disease, and losses from exposure to adverse environmental conditions over a longer period of time.

(2) A field study performed by Dekalb in 1995 indicated a trend towards reduction in CEW larval weight gain on DBT418 as compared to non-Bt corn plants. A field study conducted by Dr. Bob Lynch and Dr. Billy Wiseman, USDA-ARS, Tifton, GA also indicated a reduction in neonate and 3-day larval weight after feeding on leaf and DBT418 silks incorporated into the diet, but 3-day old larvae were not affected by fresh DBT418 silks. Six-day old larvae were unaffected by DBT418 silks, fresh or incorporated into diet. The number of larvae per ear and ear damage following CEW infestation of a DBT418 hybrid and a non-Bt control were not significantly different.

(3) In 1996-1997, Dekalb is continuing its sponsorship of work on the efficacy of DBT418 against ear feeding by CEW. Dr. Ricardo Bessin, University of Kentucky, Lexington is conducting a study to determine the efficacy of DBT 418-expressing hybrids against ear feeding by CEW. Dr. Billy Wiseman, University of Georgia, Tifton is conducting two studies involving DBT 418-expressing hybrids. In the first study, the objectives are to determine the efficacy of DBT 418 corn hybrids against ear feeding by the CEW and to measure the fitness costs for CEW found on DBT corn hybrids. In the second study, the objective is to determine the efficacy of conventionally-bred multiple borer resistant corn lines crossed with DBT 418 hybrids against ear feeding CEW. Dekalb

is also conducting internal studies regarding the efficacy of DBT 418-expressing hybrids to CEW whorl and ear feeding.

*Mycogen Plant Sciences sponsored work (Event 176-derived hybrids)*

Mycogen Plant Sciences has made no control or suppression claims on the label for CEW or other secondary target pests by Event 176-derived hybrids expressing CryI(A)b. Effects of Event 176 corn on CEW was studied by Galen Dively, University of Maryland; Randy Luttrell and D. Porter, Mississippi State University; and John Van Duyn, North Carolina State University.

(1) The North Carolina field trials conducted by Dr. John Van Duyn, North Carolina State University in 1996 indicated that there were no reductions in CEW ear feeding with Event 176 Event corn. Ear and silk feeding larvae had slightly longer developmental times on Event 176 Bt corn than on conventional corn. This delay in development on Event 176 corn will likely increase CEW larval susceptibility to pathogens, parasites, and predators.

(2) A preliminary report in 1996 from the first of a two-year study conducted by Mississippi State University trials (MSU) indicated that in silking stage corn, there were no differences in CEW growth and development (including pupal production) between CEW on Event 176 corn and non-Bt corn. The MSU work is being continued in 1997.

(3) Results from the 1996-1997 University of Maryland studies on whorl feeding CEW with Event 176 Bt corn are not yet available.

*Novartis Seeds sponsored work (BT11 and Event 176-derived hybrids)*

Novartis Seeds has made no control or suppression claims on the label for the control of secondary target pests including CEW by Event 176-derived hybrids expressing CryI(A)b on its label. Novartis Seeds has made control or suppression claims on the label for the control of southwestern corn borer, CEW, and fall armyworm by BT11-derived hybrids expressing CryI(A)b. Research on the effect of Event 176- and BT 11-derived hybrids on CEW is discussed below.

(1) Novartis Seeds reports that CEW survival on silking corn was no different on Event 176 corn hybrids and non-Bt corn hybrids. However, there may be a subtle effect on growth for CEW feeding on Event 176 corn (Novartis letter to EPA dated November 20, 1996).

(2) In 1995, Ciba Seeds conducted a field evaluation of CEW on whorl-stage Event 176 corn and non-Bt corn. Event 176 plants showed minimal leaf damage by CEW and non-Bt plants had "shot-hole" injury and elongated feeding lesions on several leaves. Such data suggest that whorl-stage Event 176 corn shows high efficacy against first generation

CEW.

(3) Research performed by Dr. Fred Gould, North Carolina State University, also showed that CEW survival was no different on silking Event 176 Bt corn hybrids and non-Bt corn hybrids. There were no statistically-significant differences in number of live larvae, larval weight, or larval development between larvae that had fed upon ears from Event 176 corn hybrids or non-Bt corn hybrids. In silking Event 176 corn, if there is selection for CEW resistance, it is subtle. Thus, Gould suggests, if there is a high dose of Bt toxin in whorl-stage corn, then Event 176 corn could act as a refuge for second generation CEW in the South. Gould indicates that Event 176-derived Bt hybrids are not appearing to exert a significant selection pressure on second generation CEW populations and may actually provide a refuge from selection for second generation CEW. However, there is still some concern about the level of selection pressure on first generation CEW feeding on whorl-stage Event 176 field corn.

The likelihood that second or later generation CEW will develop resistance to CryIA(b) as expressed in Event 176 Bt corn is considered to be substantially less than for other corn hybrids that produce Cry proteins in the silks and kernels, the primary feeding tissues for later generations of CEW. Second generation CEW typically oviposit directly on silks or ears and emerging larvae feed primarily on these tissues. Data supplied by Mycogen and Novartis indicate that second or later generations of CEW are not exposed to the CryIA(b) toxin in either the silks or the ears and therefore these generations are not undergoing a selection pressure for the development of CryIA(b) resistance. However, there is still some concern about the level of selection pressure on first generation CEW feeding on whorl-stage Event 176 field corn.

(4) Novartis Seeds is also sponsoring work in 1997 by Dr. Galen Dively (University of Maryland) to examine the performance of BT11-derived hybrids on CEW.

*Importance of Bt corn and its impact on Bt resistance management programs in areas growing corn and cotton*

Research data and model development were required on all the Bt hybrids registered to evaluate the potential impact of Bt corn on Bt resistance management programs in areas growing corn and cotton. Monsanto and Novartis were required by January 31, 1997 to submit available research data on CEW relative to resistance development and plans for producing resistance predictive models to cover regional management zones in the cotton belt based on CEW/CBW biology and cotton, corn, soybeans, and other host plants. These models must be field tested. Monsanto and Novartis submitted the available research data on CEW and plans for predictive models. Novartis, Mycogen, Dekalb, and Monsanto are sponsoring research activities regarding the development of predictive models on CEW resistance development and these are summarized below.

1. *Modification of a spatially explicit computer simulation model for predicting resistance development in CEW to Bt corn and effects of Bt corn on parameters of CEW biology and concomitant effects on population dynamics.* Monsanto, Dekalb Genetics, and investigators from North Carolina State University (Dr. John Van Duyn, Dr. Fred Gould, Dr. J.R. Bradley, and Dr. George Kennedy), Virginia Polytechnic Institute and State University (Dr. Ames Herbert), and the University of Maryland (Dr. Galen Dively) are devising strategies for developing computer simulation models which predict the evolution of resistance to CryI(A)b/CryI(A)c proteins by CEW within the corn/cotton system. Additionally, research protocols are being developed for validating model assumptions and output. Research areas include: (1) Assessing the impact of Bt corn on CEW adult emergence and oviposition in cotton; (2) Contribution of alternate hosts as refuges for CEW; and (3) Impact of Bt on CEW overwintering survival and fecundity. Information on the effects of CryI(A)b/CryI(A)c proteins on key CEW life history parameters will be integrated into the computer-based simulation model for prediction of the resistance development probability and other population biology events under different Bt crop use scenarios. EPA has limited Bt corn sales in the South due in part to a lack of biological data and simulations of population dynamics, gene flow, and resistance development estimates for CEW, a pest which moves freely from corn to cotton in the south. This research will be critical in determining appropriate long-term refuge strategies in southern areas where corn and cotton are grown in close proximity.

Data regarding CEW populations on other plant hosts (cotton, sorghum, soybean, a number of vegetables, ornamentals, and wild hosts) still remain to be gathered and analyzed by researchers.

2. *Selection on CEW by Event 176 CryI(A)b-expressing corn (sponsored by Novartis Seeds and Mycogen Corp.).* A multi-year research program is being developed by Dr. Randy Luttrell at Mississippi State University-Stoneville to examine the impact of Event 176 CryI(A)b-expressing corn on CEW in those areas where CryI(A)c-expressing cotton is grown. Results from Luttrell's 1996 field work, as others have shown in other field tests involving Event 176, indicate that Event 176 Bt corn demonstrates high efficacy towards first generation CEW and there are no measurable effects on second generation CEW.

3. *Modeling the Evolution of Resistance - population dynamics of CEW movement in corn, cotton, and other hosts involving Event 176- and BT11-derived hybrids by Novartis Seeds).* Dr. Randy Luttrell, Mississippi State University-Stoneville is examining a number of parameters that effect the evolution of CEW resistance in the corn-cotton ecosystem. The purpose of this work is four-fold: (1) Estimate impact of Bt corn on resistance evolution in corn-cotton ecosystem; vary Bt corn acreage from 5 to 100% and vary total refuge from 5 to 20% to determine the most effective strategy. (2) Simulate impact of cross-resistance between loci that confer resistance to two different toxins. (3) Simulate expression of Bt protein in whorl stage corn only, and expression in whorl and kernel to

study impact of size of refuge. (4) Simulate impact of wild hosts (density and temporal availability). The field data from research by Luttrell noted above in #2 will contribute toward demographic objectives. Validation of the genetics of the model is not possible within the two year scope of this research project. Field validation of the model would require the deliberate generation or release of a resistance population which is probably unacceptable.

#### *European corn borer biology and behavior*

A key to developing a long-term resistance management strategy of ECB on Bt corn is based on the detailed understanding of ECB pest biology and behavior. EPA required as a requirement of registration additional information regarding ECB pest biology and behavior. Key data regarding adult movement, mating behavior, gene flow, and alternate hosts of ECB are sparse. This information is valuable in designing an effective resistance management strategy that maximizes the probability that susceptible individuals arising from a refuge will find and mate with the few resistant individuals that survive exposure to the delta endotoxin produced in the Bt plant. Research efforts to assess adult movement, mating, gene flow, and survival of ECB on non-corn hosts are ongoing and are summarized below. Results from the 1997 growing season will be discussed in each registrant's annual report required to be submitted to EPA by January 31, 1998.

1. *Long range movement of adult ECB (sponsored by Novartis Seeds and Mycogen Corp.).* The purpose of this research (1996) being conducted by Drs. D. Alstad and D. Andow, University of Minnesota, is to identify the genetic structure of regional North American populations of ECB and estimate the intermating (gene flow) between these populations using isozyme electrophoretic analyses. Electrophoretic and statistical analyses of the data to estimate gene flow have not been completed. Preliminary findings indicate that little gene mixing occurred across ECB populations collected at about 250 kilometer intervals along three transects across U.S. corn production areas. The final report to be provided with the 1997 annual report, due by January 31, 1998, may indicate some useful information related to the long range movement capabilities and gene flow of ECB.

2. *Short range movement of adult ECB (sponsored by Novartis Seeds, Mycogen Plant Sciences, Dekalb Genetics, Monsanto Co.).* The purpose of this research (1996-1997) being conducted by Drs. John Witkowski and T. Hunt, University of Nebraska, is to evaluate the short-range movement of adult ECB using a mark-release-recapture technique. The objectives of this work are: (1) determine how far initial movement is away from and around a corn field, (2) determine how dispersion to and distribution among action sites change with time, (3) determine how dispersal and/or distribution differs by sex, and (4) determine how movement changes by generation. Data from 1996 field studies (first year) indicate that the gene flow between adjacent corn fields does occur. Adult movement indicated that refuge may need to be within 1500-2000 feet of Bt corn fields. Adult movement is influenced by growth stage of the corn, most adults stay

close to where they emerged, and some adults can move at least one mile within two days of emergence. These preliminary results indicate that the conventional corn refuge may need to be relatively close, but not necessarily adjacent to Bt corn.

3. *Movement of late instar ECB into Bt corn hybrids (sponsored by Dekalb Genetics, Monsanto Co., Novartis Seeds).* The purpose of this two year study (1996-1997) conducted by Dr. Rick Hellmich (Iowa State University) is to compare movement and survival of fourth instars on Bt corn hybrids expressing different levels of CryI(A)b or CryI(A)c. Late instar movement to plants expressing lower levels of Bt and survival on such plants could cause damage to the plants and reduce the effectiveness of the high dose strategy.

4. *ECB lifetable work (sponsored by DeKalb Genetics, Monsanto Co.).* The purpose of this 1996-1997 work conducted by Dr. Kevin Steffey (University of Illinois - Urbana-Champaign) is to develop a lifetable for ECB in Illinois. Information from lifestables will be used to better understand factors influencing ECB population fluctuations as well as assist in estimating parameters used in insect resistance management modeling efforts.

5. *Overwintering survival (sponsored by Novartis seeds and Mycogen Plant Sciences)* The purpose of this 1996-1997 research conducted by Dr. Blair Siegfried, University of Nebraska, is to examine the overwintering survival and CryI(A)b tolerance of ECB larvae derived from Event 176 corn. Mycogen is also conducting internal research in this area. Results from 1996 and 1997 will be pooled and discussed in the 1997 annual report due to EPA by January 31, 1998. The number of surviving larvae and the *in vitro* CryI(A)b sensitivity of populations derived from these larvae will be established and well as fitness characteristics assessed. This information will be useful in determining the significance for resistance management of ECB larvae found surviving late in the season on Event 176 corn hybrids.

#### *Laboratory-selection for ECB resistance to Bt Endotoxins*

EPA required as part of registration more information on the biology of ECB resistance and the potential for cross-resistance. Information on the nature of resistance to Bt in target pests such as the ECB are necessary to measure the effectiveness of resistance management for Bt corn. Such information is useful in evaluating which Bt endotoxins could be used in a rotational or pyramiding scheme. Currently this work is limited to working with laboratory-selected populations since resistance in the field has not been detected. Laboratory studies selecting for ECB-tolerant strains provide information on the genetic potential of ECB to develop resistance, but are not conclusive on whether resistance will develop in ECB populations under field conditions. Bt corn and ECB in the field may pose a different situation than larvae feeding on Bt insecticides in a laboratory diet under controlled conditions. Laboratory-selected tolerant colonies, nonetheless, will be useful in experiments concerning the mechanism of resistance and the genetic basis for resistance, and provide information on the potential for cross-resistance

between Bt toxins. Research (1996-1997) on ECB colonies selected for resistance in the laboratory to CryI(A)c and CryI(A)b are described below.

1. *Effect of CryI(A)c resistance on ECB fitness* (research by Dr. William Hutchison, University of Minnesota and sponsored by Novartis Seeds and Mycogen Plant Sciences). Multiple CryI(A)c-resistant ECB colonies were developed in the laboratory. These colonies were selected for tolerance to a non-viable formulated *Pseudomonas fluorescens* engineered to express the CryI(A)c endotoxin (formulated MVP (Mycogen Corp.)) under acute exposure conditions. Selection pressure was removed from these colonies and fitness costs were estimated. Results show that after nine generations of no exposure to CryI(A)c, there was no difference in susceptibility between the selected and non-selected colonies. Thus, Hutchison concluded that there was a significant fitness cost associated with the development of CryI(A)c tolerance in these laboratory colonies. Additional studies on other fitness parameters including fecundity, developmental rate, survival, and larval weights are being conducted in 1997.

2. *Susceptibility of CryI(A)c resistant ECB to other Cry proteins (Cross-resistance)* (research by Dr. William Hutchison, University of Minnesota and sponsored by Novartis Seeds and Mycogen Seeds). In laboratory binding studies, CryI(A)b and CryI(A)c delta endotoxin are structurally similar and appear to bind to the same midgut epithelial receptor in ECB (Denolf *et al.*, 1993). The structural and target site similarities of the two delta endotoxins suggest that insects developing resistance to one delta endotoxin would likely develop resistance to the other. Research by Dr. Blair Siegfried tested the estimated LC<sub>99</sub> derived from the dose-response curve to CryI(A)b against the CryI(A)c resistant ECB strains developed at the University of Minnesota. These strains do not appear to be resistant to CryI(A)b. Resistance to CryI(A)c is not conferred to the CryI(A)b delta endotoxin. The CryI(A)c resistant strains were tested for cross-resistance to other Cry proteins. The results of single dose diet surface bioassays for 17 different single Cry protein preparations suggest that cross resistance is not present for several toxins, i.e., CryI(A)b, Cry7(A)b, and PS28(C), and is present for others, i.e., CryI(B), CryI(F)a. These CryI(A)c resistant ECB strains show a very narrow range of cross-resistance. Additional dose response determinations on formulated products are continuing in 1997.

3. *Laboratory selection experiments for ECB CryI(A)b resistant colonies.*

(a) Dr. Keil, University of Delaware (Novartis Seeds sponsored work), selected for a CryI(A)b-resistant ECB colony under acute exposure conditions in the laboratory. Keil notes there are fitness costs associated with the development of CryI(A)b tolerance, including temporal changes in development, reduced pupal weight, as well as reduced egg deposition in the selected colony. CryI(A)b-tolerant larvae failed to cause any leaf feeding damage on Event 176-derived corn expressing the CryI(A)b toxin. There was no difference in feeding damage between the CryI(A)b-tolerant larvae and non-selected

CryI(A)b larvae on Event 176-derived corn. These results indicate that the gene(s) involved in tolerance to CryI(A)b in the laboratory-selected colonies do not confer any increased tolerance to Event 176-derived corn plants that express the CryI(A)b toxin.

(b) Pioneer Hybrid International has selected for two CryI(A)b-tolerant ECB colonies under chronic exposure conditions in the laboratory (Lang *et al.*, 1996). Chronic exposure more closely mimics field exposure conditions for ECB larvae. Following 13 generations of selection pressure, neither colony exhibited any increase in feeding damage on CryI(A)b-expressing corn plants compared to non-selected larvae. Neither selected colony was able to survive exposure to Bt concentrations (in an artificial diet) that approached those concentrations that would be encountered in a Bt corn plant.

(c) Dr. Blair Siegfried (University of Nebraska) and Dr. Randy Higgins (Kansas St. University) have created two additional CryI(A)b-selected ECB colonies for study. Novartis Seeds established a research collaboration with Siegfried's laboratory in 1997 to evaluate these colonies for cross-resistance to other Cry toxins, fitness parameters, and leaf feeding behavior on CryI(A)b-expressing corn plants. Dr. William Hutchinson (University of Minnesota) and Mycogen Plant Sciences are both initiating efforts to select for CryI(A)b resistance in ECB to facilitate further research on the biology of resistance.

4. *Midgut binding studies* (research by Dr. William Hutchinson, University of Minnesota and sponsored by Mycogen Seeds). Midgut binding studies were expected to be initiated in 1997. The Cry delta endotoxin binds to a specific receptor on the midgut lining and the cells rupture. These studies will examine whether modification in ECB midgut binding may be the mechanism of resistance.

### *Effective Refuges*

All available evidence supports the conclusion that a "structured" refuge is a necessary component of a successful long-term resistance management strategy and that the success of the high dose strategy will be compromised if there is no effective refugia. Stakeholders have hotly debated the size and deployment of a refuge for Bt corn. Prior to the August 1995 registration of the first CryI(A)b delta endotoxin in corn, a few entomologists recommended a 5 to 50% structured refuge, but no consensus had been reached with industry, EPA, USDA research and extension scientists, growers, and other stakeholders at that time. In 1995, it was thought that based on market penetration estimates there would be enough non-Bt crop acreage to serve as a viable refuge in the first five years following full-scale commercialization. A "structured" refuge requirement on the registrations thus would not be necessary. However, the Agency mandated as a condition of registration that research data be collected to develop an effective refuge with both temporal and spatial refuges to be investigated. In addition, a draft refuge strategy must be submitted to the Agency by August 9, 1998 and a final refuge strategy submitted by January 31, 1999. The registrant must implement an EPA approved "structured" refuge plan or an EPA approved alternative resistance management plan no later than April 1, 2001. Registrants are required to



discuss the development and implementation of the refuge plan and alternative resistance management practices with EPA throughout the development and the implementation. EPA also required as a part of the registration for all Bt corn products specific monitoring and remedial action if any resistance occurs. The registrations indicate that if remedial efforts are not effective in mitigating resistance, the registrant will voluntarily cease sale of all corn hybrids that contain the Bt corn plant-pesticides in the county experiencing loss of product efficacy and the bordering counties until an effective local management plan approved by EPA has been implemented. EPA can also halt the future sale of Bt corn plant-pesticides if resistance occurs.

In 1996 and 1997, less than 5% of the total corn acreage was planted with Bt corn hybrids and of this acreage, the density in any county or state was low. However, there may be pockets of contiguous Bt corn acreage with the refuge acreage too far away to provide an adequate supply of susceptible insects to mate with any potentially Bt-resistant insects that emerge. This is the contention offered by a review article of Alstad and Andow (1996). They conclude that structured refuges make as much biological sense at the outset as they do three years following the initial full-scale commercialization even if there is a lack of scientific information to describe the optimum size of a refuge.

In 1997, as a result of multi-stakeholder discussions at the USDA Bt Resistance Management Forum (April 1996) and the USDA NC-205-led Consortium meetings held in 1995-1997, a more consensus viewpoint on refuge is beginning to emerge. This emerging consensus focuses on what constitutes an effective refuge size, i.e., how many mating stage adults are produced in the right place at the flight time to mate with adults emerging from Bt producing varieties rather than whether a “structured” refuge should be required. The recommendations of the USDA NC-205 led consortium have been recently published in a report (NCR 602) entitled “Bt Corn & European Corn Borer - Long-Term Success Through Resistance Management” (Ostlie *et al.*, 1997). This report recommends having a “structured” refuge which is 20 to 30% non-Bt corn to prevent Bt delta endotoxin exposure to 20 to 30% of the larval population. In continuous corn acreage sprayed with insecticides, the refuge size would be increased to perhaps 40% to compensate for larval mortality. Where there are many alternate hosts that do not contain Bt proteins, a smaller refuge may be suitable. This reduction in refuge size assumes that ECB from alternative hosts emerge at similar times as ECB from corn. At present, the knowledge base is still limited as to what proportion of the local ECB population flows through non-Bt hosts. More information is needed on the synchronous emergence from refuges and Bt crops. Therefore, as a baseline, the USDA NC-205 publication recommended that a 20 to 30% non-Bt corn refuge may be the simplest and best way to insure delayed resistance. This publication indicated that the actual amount of refuge required will vary among regions, farms, and corn production system. Therefore, growers should contact local extension entomologists for specific refuge recommendations.

At present, Monsanto and Dekalb Genetics are the only two registrants that mandate a particular structured refuge through their Technology Management (Grower) Agreements. A grower purchasing from Monsanto or Dekalb has two options: a 5% unsprayed non-Bt refuge or a 20%

sprayed non-Bt refuge. These two refuge options are also stated in their respective grower guides. In 1996 and 1997, Mycogen Plant Sciences's and Novartis Seeds's Grower Guides/Technical Bulletins indicated their commitment to development of long-term resistance management strategies through the support of research efforts, but neither mandate nor recommend a particular refuge option. Mycogen's 1996 and 1997 technical bulletins for Event-176 corn hybrids indicate a commitment to develop a long-term resistance management strategy, provide general resistance management guidance, and recommend that not all corn acres be planted in Bt corn. Novartis Seeds's 1996 and 1997 technical bulletins for Event-176 corn hybrids indicate a commitment to develop a long-term resistance management strategy, provide general resistance management guidance, and indicate that part of a long-term resistance management strategy may be "the maintenance of a refuge where susceptible populations of ECB can escape exposure to the insect control protein in Maximizer hybrids." Novartis Seeds for its BT 11 hybrids "encourages" growers to: (1) plant Bt hybrids in large block, (2) scout for non-target pests and use IPM strategies, (3) maintain a refuge of non-Bt corn, and (4) monitor for unexpected levels of insect damage in Bt corn. On November 13, 1997, Novartis Seeds announced that in 1998, they would recommend that growers follow the guidelines outlined in the North Central Regional Publication 602 (Ostlie *et al.*, 1997). As stated earlier, the NCR 602 report recommends having a "structured" refuge which is 20 to 30% non-Bt corn to prevent Bt delta endotoxin exposure to 20 to 30% of the larval population. In continuous corn acreage sprayed with insecticides, the refuge size would be increased to perhaps 40% to compensate for larval mortality. Where there are many alternate hosts that do not contain Bt proteins, a smaller refuge may be suitable. Again, the report indicates that the actual amount of refuge may vary between regions, farms, and corn production systems.

Mycogen has indicated in its 1996 year-end report that in some of the verbal presentations to growers they are supporting the recommendations from the USDA NC-205-led consortium on Bt corn resistance management. They recommend planting at least 10 to 40% of a grower's total corn acres with a conventional corn hybrid and to use the high end of this range as a minimum if insecticides are used to control ECB infestations and the low end if no insecticides are used to control ECB infestations. Other ECB-susceptible crops may be used such as oats, peppers, popcorn, potatoes, snap beans, sorghum, and sweetcorn. The ECB-susceptible crop should be planted close to the Bt corn to reduce the chances for an isolated population of Bt-resistant borers to develop. The recommended arrangements for the Bt corn and ECB-susceptible crops are to: (1) Plant in two blocks of Bt and non-Bt corn in the same field; (2) Plant Bt and non-Bt corn in adjacent fields, or; (3) Rotate Bt corn fields to a ECB-susceptible crop in the next growing season. Mycogen indicates to growers that mixing conventional and Bt corn hybrids in the planter box or splitting the planter to sow alternating strips in the field is not recommended.

Several research efforts begun in 1996 and continuing in 1997 are aimed at determining the size and deployment of an effective refuge strategy. Research progress and a summary of the results from the 1997 studies discussed below will be submitted by the registrants to EPA by January 31, 1998. Efforts to date and 1997 research plans are summarized below.

1. *Structured early/middle/late planting time strategy.* In 1996, Dr. Marlon Rice and collaborators at Iowa State University (Novartis Seeds and Mycogen Corp. jointly-sponsored research) conducted field research on a block-design planting strategy of Bt and non-Bt corn, with a fixed amount of refuge (33%). In addition, this strategy involved a temporal planting scheme so that non-Bt corn was interspersed between an early and late-season planting of Bt corn. It is thought that the non-Bt corn could escape the most dense moth flights yet still support sufficient ECB populations to act as mating partners to any resistant ECB that may be selected in the Bt corn plots. The advantages of this strategy to growers, if it is effective, is that they will have to purchase less higher-priced Bt corn seed and they will encounter less yield loss in the non-Bt corn plot. Results from this type of study may help farmers decide on where and when to place Bt hybrids in fields at greatest risk to ECB infestations. This type of refuge strategy might be an effective option for resistance management. Alstad and Andow (1995) discussed the advantages of this approach and modeled potential outcomes in a recent review.

In 1996, the first year of this two year study, the results indicate that adult ECB females laid the same number of eggs on Bt corn as on conventional hybrids, regardless of the planting date or ECB brood. Stalk tunneling information indicates that ECB survived on conventional corn planted in close proximity to Bt hybrids. More definitive information will be available after the completion of the second year of the study in 1997.

Additional 1997 studies funded by Novartis Seeds on temporal/spatial refugia are being conducted by Dr. Ken Ostlie (University of Minnesota), Dr. Murdick McLeod (South Dakota State University), and Dr. Dennis Calvin (Pennsylvania State University; refer to #4 below).

2. *Magnitude and mechanism of "Halo Effect" near Bt corn (sponsored by Novartis Seeds, Mycogen Plants Sciences, Dekalb Genetics, Monsanto Co.).* Research conducted by Drs. D. Andow and D. Alstad, University of Minnesota, indicate that ECB infestations are reduced in conventional corn fields within 150 ft. blocks of commercial size plantings of Bt fields. This research supports the finding above that conventional corn planted in close proximity to Bt corn will support susceptible ECB populations, although perhaps at a somewhat reduced level than if conventional corn were planted further away from Bt corn. It also suggests that ECB movement is limited between Bt and non-Bt fields so that the likelihood of mixing of susceptible with resistant adults is greater if conventional corn is planted in close proximity to Bt corn hybrids. Mycogen Plant Sciences, Dekalb Genetics, Monsanto Co. and Novartis Seeds are continuing to support research on the halo effect in 1997 to: (1) determine the benefits for conventional corn within the halo, (2) determine how halo dynamics are affected by the size and layout of the refuge and Bt units, and (3) determine the mechanism of the halo effect.

3. *Survival of ECB on non-corn hosts (sponsored by Novartis Seeds, Mycogen Plant Sciences, Dekalb Genetics, Monsanto).* The purpose of this research (1996-1997) being

conducted by Dr. Rick Hellmich, USDA/ARS, Iowa State University is to examine the survival of ECB on non-corn and weed hosts. Preliminary results indicate that weed hosts produce less than 1% of ECB and thus weeds will not be a sufficient ECB refuge to be useful in resistance management. Sorghum, some small grains, and some other non-corn crops may serve as effective refuges, but there isn't sufficient information to quantify the refuge value of the non-corn crops and make specific recommendations at this time. The value of non-corn crops as refuges is particularly important in crop rotations that include non-corn ECB host crops.

4. *Sequential plantings of field corn as temporal refugia for ECB (sponsored by Dekalb Genetics, Monsanto Co., Novartis Seeds)*. The purpose of this two year study (1996-1997) conducted by Dr. Dennis Calvin, Pennsylvania State University, is to evaluate the value of temporal refuges as a resistance management strategy as well as the value of non-transgenic corn patches in a landscape design. The objectives include: (1) quantifying egg mass recruitment, larval survival, and population stage structure in sequentially planted field corn, and (2) evaluating the relative attractiveness of various plant development stages to ECB females.

5. *Optimization model for ECB incorporating resistance management factors including refuge (sponsored by Dekalb Genetics, Monsanto Co., U.S. EPA (Contract CR822045-01-4))*. The purpose of this 1997 research to be conducted by Dr. Rick Hellmich, Dr. Terrance Hurley, and Dr. Bruce Babcock (Iowa State University) is to develop an economic model which examines the profitability of using a refuge. If alternate crops are good ECB producers, insect resistance management using crop combinations could go hand-in-hand with net profits. These researchers developed an economic model of pest management with pest resistance to estimate the constant proportion of refuge that maximizes farm income over a fixed planning horizon (Hurley *et al.*, 1997). Results indicate that there is a clear economic tradeoff between pest control and population management benefits afforded by a Bt corn variety and the resistance management benefits and savings in production costs afforded by refuge. From this model, the researchers concluded that, under certain circumstances, a 20 to 40% refuge is economically sensible. This model could also be used to factor in cultural practices such as disking that reduce ECB numbers.

6. *Evaluation of resistance management strategies for Bt-corn (sponsored by Dekalb Genetics, Monsanto Co., Novartis Seeds)*. The purpose of this 1997 research to be conducted by Drs. Bushman, Higgins, and Sloderbeck, Kansas State University, is to evaluate ECB production and practical utilization of several refuge planting options and to evaluate the practical utilization of neighborhood suppression effects of ECB as suggested by the "halo effect" associated with planting Bt corn hybrids near conventional hybrids.

7. *Impact of Yieldgard (Bt-11 and MON810) Bt corn hybrids on management of ECB and other corn insects (Dekalb Genetics, Monsanto Co., Novartis Seeds)* The purpose of

this 1997 research to be conducted by Mason and Keil, University of Delaware, Whalon, Michigan St. University, and other researchers is three-fold: (1) evaluate effectiveness of other commonly grown host crops in the Mid-Atlantic region as refuges for non-Bt selected ECB; (2) assess the association between behavioral and physiological effects on a Delaware population of ECB; and (3) evaluate the effectiveness of Bt corn hybrids in controlling other corn insect pests including CEW, fall armyworm, true armyworm, and southern cornstalk borer. This research will provide information related to appropriate refuge sizes for the Mid-Atlantic region as a result of better quantifying the contribution of non-corn refuges.

8. *Grower Surveys - Attitude toward refuges (sponsored by Novartis Seeds and Mycogen Plant Sciences)*. Two grower surveys were conducted by entomologists, Dr. John Witkowski, University of Nebraska and Dr. Marlon Rice, Iowa State University, in 1996. Based on these first surveys, growers would prefer a rotation option over planting Bt and conventional hybrids in a specified ratio in field to field patchwork or in-field refuges. The results of these surveys are important in determining what grower acceptance is likely to be in adoption, implementation, and effectiveness of a refuge. This type of survey also provides insight as to possible research priorities for refuge research.

These type of grower surveys was to be expanded from two to six in 1997 to cover additional corn growing areas especially in the Midwestern corn belt. The following researchers will be conducting grower surveys: Dr. Marlon Rice, Iowa State University, Dr. John Witkowski, University of Nebraska, Dr. Randy Higgins, Kansas State University, Dr. Kevin Steffey, University of Illinois, Dr. Dennis Calvin, Pennsylvania State University, Dr. Ken Ostlie, University of Minnesota.

9. *Impact of Bt corn events on CEW and its implications to resistance and population suppression in soybeans (Dekalb Genetics and Monsanto Co.)* The purpose of this 1997 research to be conducted by Dr. Galen Dively and collaborators, University of Maryland, and Dr. Ames Herbert, Virginia Polytechnic Institute and State University, is to quantify the mortality and behavior of CEW feeding on Bt corn hybrids and investigate the reproductive performance of surviving moths and their role in resistance development and colonization of nearby soybean fields. This work will improve our understanding of whether soybean fields are impacted by CEW moving from Bt corn hybrids to soybeans.

#### *Surveillance and Tracking (Monitoring)*

EPA required as part of registration a monitoring plan including the development of ECB baseline susceptibility responses, development of a discriminating concentration (i.e., bioassays that use one optimal or nearly optimal dose, i.e., a discriminating or diagnostic dose concentration, to distinguish between susceptible and resistant individuals) to detect changes in ECB sensitivity, routine surveillance, and remedial action if there was suspected resistance. The purpose of monitoring is to learn whether a field control failure resulted from resistance or other factors, e.g.,

factor that might inhibit expression of the Bt Cry delta endotoxin by the plant. The extent and distribution of resistant populations can be mapped and alternative control strategies implemented in areas in which resistance has been documented. It may also be possible to detect resistance before it happens and control failures occur, if monitoring techniques are sensitive enough to discriminate between resistant and susceptible individuals. Surveillance by growers is essential. The NC-205 publication NCR 602, "Bt-Corn & European Corn Borer: Long-Term Success Through Resistance Management" (Ostlie *et al.*, 1997), discusses how Bt corn can be used to manage ECB and how to use this technology for long-term profitability. This publication provides specific resistance management recommendations including monitoring for first and second generation ECB. Current monitoring activities are summarized below.

*1. Baseline Susceptibility and Development of Diagnostic Bt Concentrations for Monitoring (Dr. Blair Siegfried, University of Nebraska, sponsored by Mycogen Plant Sciences, Novartis Seeds, Northrup-King/Novartis Seeds, Monsanto Co., Dekalb Genetics Corporation).* This work is designed to develop techniques for monitoring Bt resistance in field populations of ECB. Baseline responses to Cry 1(A)b have been determined for a number of ECB populations collected from across the corn belt over the last 3 years. Results of baseline susceptibility studies conducted on Nebraska ECB populations (Siegfried *et al.*, 1997) indicated that distinct geographic populations showed a greater than 5-fold, based on  $LC_{50}$ , variability in their responses to the CryI(A)b toxin. The toxin was obtained from a fermentation run of *Bacillus thuringiensis* Berliner Subsp. *kurstaki* (HD1-9 strain) that produces only the CryI(A)b protein. Baseline susceptibility of ECB using a commercial formulation of *Bacillus thuringiensis* Berliner Subsp. *kurstaki* (Dipel ES) was also conducted by Kansas researchers using Kansan and Iowan field populations (Huang *et al.*, 1997). These researchers found regional differences in susceptibility to Dipel. Siegfried *et al.* (1997) and Huang *et al.* (1997) indicate that the level of susceptibility reported in their studies may not accurately reflect the susceptibility of field ECB populations because the number of insects used to start the colonies was small and may not be representative of field populations.

Studies in 1996 attempted to develop a diagnostic concentration based on baseline susceptibility studies conducted in 1995. Experiments were also designed to validate the  $LC_{99}$  using field populations of ECB. Baseline responses to CryI(A)c are also being conducted.

Larvae were collected from a total of eight sites in seven states (i.e., Nebraska, Indiana, Illinois, Pennsylvania, North Carolina, Iowa, Minnesota) where Bt corn hybrids had been planted. Dose-response bioassays were performed using neonate larvae on artificial diet containing purified CryI(A)b protein. Results indicated that the difference in the  $LC_{50}$  values between the ECB populations were similar to those observed during 1995 and represent natural variability. An estimated diagnostic CryI(A)b concentration,  $LC_{99}$ , was estimated from these baseline data. This work was being continued in 1997. Mycogen also conducted standard bioassays of field collected ECB in-house in 1997.

The estimated diagnostic LC<sub>99</sub> concentration was used to detect changes in the sensitivity of ECB populations that have been exposed to Bt in 1996. When the eight ECB populations were exposed to the estimated LC<sub>99</sub> concentration, 99-100% mortality was observed. There were differences among the populations tested, but these results support the conclusion that there was no change in susceptibility of ECB to the CryI(A)b toxin. The LC<sub>99</sub> mortality value is a more useful diagnostic concentration for estimating ECB susceptibility levels rather than the EC<sub>99</sub> growth inhibition value. However, there are significantly higher levels of Bt toxins expressed in Bt corn meaning that the estimated LC<sub>99</sub> is probably several fold lower than what the ECB will be challenged with (i.e., overall concentrations in the field will be higher) in Bt corn fields. Thus, the estimated LC<sub>99</sub> will distinguish between resistant and susceptible individuals and can be used in a diagnostic bioassay for Bt resistance detection.

To further validate the diagnostic concentration, it will be necessary to develop a CryI(A)b resistant ECB strain. The estimated LC<sub>99</sub> has been tested against the CryI(A)c resistant ECB strains developed at the University of Minnesota. However, these strains do not appear to be resistant to CryI(A)b. Additional studies with CryI(A)b resistant ECB will be required to confirm the efficacy of the estimated CryI(A)b LC<sub>99</sub> as a discriminating dose. These studies will be conducted when a resistant CryI(A)b strain becomes available.

2. *Establishing CEW baseline susceptibility to CryI(A)b.* Dekalb Genetics/Monsanto Co. are sponsoring the research by Ricerca to establish CEW baseline susceptibility to CryI(A)b and to determine a diagnostic dose. This work is important in assessing changes in CEW susceptibility as a result of exposure to CryI(A)b. The project will be done in cooperation with the current monitoring program for CryI(A)c coordinated by Dr. Hardee, USDA/ARS, Stoneville, MS (see description under Section IV. Bt cotton resistance management).

3. *Surveillance and Remedial Action.* As part of the requirements of each Bt corn plant-pesticide registration, each registrant is required to carry out surveillance and implement a remedial action plan if there are incidents of confirmed resistance. Each company has instructed its customers to have regular surveillance programs and report any unexpected levels of ECB and CEW damage via a toll-free customer service number. Each company will investigate and identify the cause for this damage by local field sampling of the plant tissue and suspect insect populations followed by appropriate *in vitro* and *in planta* assays. Confirmed incidences of resistance are required to be reported to the Agency within 30 days and appropriate remedial action is required to mitigate ECB and/or CEW resistance. Within 90 days of a confirmed instance of ECB and/or CEW resistance, the registrant will: (1) notify the Agency of the immediate mitigation measures that were implemented, and (2) submit to the Agency a proposed long-term resistance management action plan for the affected area, (3) work closely with the Agency in assuring that an appropriate long-term resistance management plan for the affected area is implemented,

and (4) implement an action plan that is approved by EPA and that consists of some or all of the following elements: (a) informing customers and extension agents in the affected areas of ECB and/or CEW resistance, (b) increasing monitoring in the affected area, and ensuring that local ECB or CEW populations are sampled on an annual basis, (c) recommending and implementing alternative means to reduce or control ECB or CEW populations in the affected areas, and (d) implementing a structured refuge in the affected areas based on the latest research results. The implementation of a refuge strategy will be coordinated by the Agency with other registrants. If the above elements are not effective in mitigating January 12, 1998 resistance, the registrant will voluntarily cease sale of all corn hybrids that contain the particular Bt corn plant-pesticide in the county experiencing loss of product efficacy and the bordering counties until an effective local management plan approved by EPA has been implemented. During the voluntary suspension period, the registrant may sell and distribute in these counties only by obtaining EPA approval to study resistance management in those counties. The implementation of such a strategy will be coordinated by the Agency with other registrants. Seed lot purity will influence the precision required to detect ECB resistance. Industry cooperation with extension entomologists is considered important in communicating specific information on definitions of “unexpected damage.” This topic has been discussed in multi-stakeholder meetings such as the USDA/ARS NC-205-led meetings on Bt corn resistance management and the USDA Bt resistance management forum.

Mycogen investigated three customer calls in 1996 related to incidents of unexpected levels of ECB and determined that none of these was related to CryI(A)b resistant ECB. Two of the calls were from growers who forgot where the Event 176 hybrid corn had been planted and one came from a crop consultant who misidentified common stalk borer feeding for ECB.

### *Grower Education*

All registrants are required, as a part of registration, to implement a grower education program and develop Grower Guides which will include current information regarding insect resistance management and integrated pest management. Amongst all stakeholders, there is universal agreement that grower acceptance and adoption of insect resistance management strategies are critical to the success of Bt corn. In a 1996 grower survey underwritten by Novartis Seeds and Mycogen Seeds, the majority of growers who responded indicated that they rely on an industry representative for their product information. Therefore, industry has a primary responsibility to provide accurate and effective information regarding insect resistance management to the grower. Other stakeholders, such as academic and extension entomologists, county agents, crop consultants, USDA and EPA share this responsibility.

Both Novartis Seeds and Mycogen Plant Sciences reported in their 1996 annual reports that an extensive grower education program has been implemented. Grower Guides are mailed to each grower and information tags are affixed to each bag of seed describing appropriate insect



resistance management information. Both companies participate in the NC-205-led consortium discussion on Bt corn resistance management. Mycogen Plant Sciences and Novartis Seeds noted that they incorporated the specific recommendations for growers on resistance management developed by the NC-205-led consortium and put them in their written materials and slide presentations. These recommendations will now be part of Novartis Seeds 1998 Grower Guides for BT-11 and Event 176-derived hybrids. Both companies report they give numerous presentations to growers, agriculture media, university extension and crop consultants on product performance and insect resistance management and participate in multi-stakeholder forums to discuss resistance management. Novartis regulatory affairs group hosted seminars on resistance management issues with Novartis Seeds sales, marketing, and agronomic staff.

#### *Development of products with alternative modes of action*

Industry is developing other corn lines that involve the expression of novel Bt genes acting by mechanisms different from currently registered Bt genes. These novel genes could be combined with currently registered Bt genes, insecticidal genes with mechanisms of action different from Bt, and inherent host plant resistance traits as a means for combating the development of ECB resistance to either CryI(A)b or Cry(A)c delta endotoxin expressed in corn. Pyramiding or stacking genes with different modes of action is advocated by entomologists as a powerful tool to mitigating the development of resistance. Roush (1994) has modeled the effects of pyramiding and results indicate that resistance may be delayed by greater than 1000-fold.

#### *Bt corn impact on mycotoxins and plant diseases*

Mycotoxins are secondary metabolites produced by some fungi that contaminate food or animal feed. Monsanto is funding research to examine mycotoxin reduction in Yieldgard ears and determine to what extent Yieldgard hybrids affect mycotoxin levels in grain as a result of reduced insect injury in ears. This work is being performed by two research groups: 1) Munkvold, Hellmich, Showers, and Rice from Iowa State University and 2) Herbert (Virginia Polytechnic Institute) and Dively (University of Maryland). Reduced mycotoxin levels would be an additional benefit of the use of Bt corn to humans and animals. Reduction in mycotoxin levels is also an important IPM consideration for the use of Bt corn hybrids.

Novartis Seeds is also funding internal research to examine to what extent Event 176 and BT11 hybrids affect mycotoxin levels in grain.

#### *Public Comments*

Of the 100 comments received as a result of the two public hearings held on Bt plant-pesticide resistance management this year, about 15 specifically focused on Bt corn resistance management issues. A discussion of the public hearings is found above in Section I of this paper.

Comments came primarily from two groups: academic and extension entomologists and

industry/seed companies utilizing the Bt corn hybrid technology. The comments focused on whether resistance management plans should be mandatory or voluntary and on the scientific needs for resistance management plans. Industry/seed companies believe that resistance management plans should be voluntary and are the responsibility of product stewardship efforts by industry. Academic and extension entomologists, with one exception, believe the resistance management plans should be mandatory for Bt plant-pesticides produced in corn. All parties agree that additional data need to be gathered to develop a long-term resistance management strategy. Research in the following areas was generally described as necessary: pest biology, genetics, behavior, and ecology, population dynamics, gene flow, refuge strategies, biology of resistance and cross-resistance, high dose effectiveness on primary and secondary pests, and importance of Bt corn and its impact on Bt resistance management programs in areas growing corn and cotton. Monitoring (surveillance and tracking) and grower education efforts are essential.

However, there were three comments from the University of Missouri, Texas Corn Growers Association, and Northrup-King/Novartis Seeds who felt that the blanket sales restriction in the South on Bt corn hybrids with Bt expression in silks and kernels (i.e., MON810, BT11, DBT418) in parts of Missouri and Texas should be removed because it is economically more desirable to plant Bt corn than Bt cotton. In Texas, the desire is to plant Bt corn hybrids with silk and kernel expression in counties north and west of Lubbock, i.e. in the Texas Panhandle. In Missouri, the desire is to plant Bt corn hybrids with Bt expression in silks and kernels in the Bootheel region of Missouri. The comments from both states report that it is unlikely that Bt cotton would be grown in these regions. However, according to the registration for Bt cotton (issued prior to the registrations for BT 11 and MON 810 corn registrations), Bt cotton can be grown in the Texas Panhandle and the Missouri Bootheel. In addition, non-Bt cotton could still be grown in some of these counties. Based on the discussion of Cry effects on CEW movement above, there would be some selection on CEW/CBW occurring if Bt corn hybrids with silk and kernel expression were planted in these areas. Any Bt cotton and Bt microbial sprays used on cotton or vegetable crops in close proximity to Bt corn hybrids would add further selection pressure toward the development of Bt resistance. If no cotton/ Bt cotton or other vegetable crops using Bt microbial sprays were grown in these selected counties in the Missouri Bootheel or Texas Panhandle, then the selection pressure would be very much reduced for the development of resistance to later generations of CEW/CBW.

### *Summary*

Prior to the registration of the first Cry delta endotoxin produced in corn in August 1995, there was no consensus on the size and structure of an effective refuge and the incentives needed for grower adoption. Figures ranged from 50% non-Bt structured refuge needed to 0 to 5% refuge needed. EPA determined that more information was needed to support specific refuge options and required as part of registration research to develop the size, structure, and deployment of a refuge and implementation of a refuge. However, EPA believed that during the first five years following commercialization (approximate time-limit of the conditional registrations for Bt corn),

there would not be enough Bt corn acreage to provide substantial Bt selection pressure for the development of ECB resistance. Consequently, EPA did not mandate any specific refuge requirements. However, EPA identified several research areas in which data were needed to develop a long-term resistance management strategy: (1) information on ECB pest biology and behavior, (2) feasibility of refuge options, (3) development of discriminating dose concentration assay, (4) effect of corn producing the CryI(A)b or CryI(A)c delta endotoxin on pests other than ECB including CEW, (5) the biology of ECB resistance and cross-resistance. Research progress in all of these areas will be reported to the Agency in each registrant's annual report due January 31, 1998.

EPA required annual reporting of sales information for Bt corn as a requirement of registration. Approximately 400,000 acres (about 0.5% of the total corn acreage) of Bt corn were planted in 30 states in the U.S. in 1996 and approximately 3.5 to 4 million acres (about 5% of the total corn acreage) is expected to have been planted in 1997. Based on a three-year average, there are approximately 70 to 80 million acres of corn planted annually in the U.S.

Based on efficacy research submitted to the Agency, Bt corn hybrids constitutively expressing the CryI(A)b and CryI(A)c delta endotoxins throughout the plant, including silks and kernels, provide an effective high dose for both first and second generation ECB. The registered plant-pesticides in this category are from MON810, BT11, and DBT418 transformation events. Event 176 Bt corn hybrids, express the CryI(A)b delta endotoxin in green tissue and pollen, and provide an effective high dose in whorl-stage corn for first generation ECB control, but provide only about 70 to 75% control of second generation ECB in silk-stage corn. Research and commercial plantings to date indicate that both types of Bt corn hybrids control ECB better than conventional insecticides. Additional ECB efficacy research is supported by all Bt corn registrants.

Research results indicate that there is significant short-range ECB adult movement indicating that it might be more effective to construct the non-Bt refuge in close proximity to Bt acreage. Alternative crops may serve as effective refuges, but more information is needed. More information on weedy hosts is needed to determine whether they can serve as effective refuges. Based on Alstad and Andow's simulation model (Alstad and Andow, 1996), an unsprayed 5% non-Bt refuge may not be adequate for ECB resistance management. Refuge size may vary depending on whether insecticides will be used and whether there are alternative hosts in close proximity to the Bt corn acreage planted. Early research results indicate that a structured early/middle/late planting time strategy may also be a practical and effective refuge strategy. More definitive information will be available after the 1997 growing season to assist in developing a long-term resistance management strategy for Bt corn.

Research on the biology of resistance has led to the development of CryI(A)b and CryI(A)c-tolerant colonies of ECB. Insects resistant to one of these Cry proteins show no cross-resistance to the other Cry protein. However, laboratory-selective conditions are not often representative of field-selective conditions. Laboratory studies selecting for ECB-tolerant strains provide information on the mechanism of resistance in ECB, but do not predict whether resistance will

develop under field conditions.

EPA required routine monitoring and an annual monitoring report as part of registration for Bt corn. In 1996 and 1997, the EPA received no reports of putative ECB or CEW resistance to CryI(A)b or CryI(A)c. Baseline susceptibility studies have indicated that there is a range of variability in geographically distinct populations, but these studies have not indicated any significant changes in ECB susceptibility over time. A discriminating concentration,  $LC_{99}$ , has been developed to detect changes in ECB sensitivity and its efficacy has been tested. Preliminary results indicate that it can be used for routine surveillance and as a trigger for remedial action. Research is continuing on baseline susceptibility and testing the discriminating dose concentration.

Although the primary focus is on ECB, there is also a concern about the potential development of Bt resistance in CEW. The development of CEW resistance to Bt produced in crops could negatively affect the utility of Bt cotton and Bt microbial sprays on vegetables and other crops. Research efforts to date indicate that silk and kernel expression in Bt corn hybrids will likely increase the selection for CEW resistance especially in cotton-growing areas. Silk and kernel expression is found in constitutively expressing Bt corn hybrids, i.e., events MON810, DBT418, and BT11. In general, laboratory and field studies indicate that CEW development was delayed and larval weight was reduced by the presence of Bt in the plants, although the magnitude of the difference varied among Bt hybrids and across time for the three events tested, MON810, BT11, and Event 176. The greatest developmental delays and weight losses were observed in BT11 and MON810 corn hybrids. There appears to be no selection for Bt resistance in CEW in Event 176 Bt corn hybrids and these types of hybrids may actually provide a refuge from selection for second generation CEW in the South. There is a sufficiently high dose expressed in whorl-stage Event 176 corn to control first generation CEW. Additional research efforts are underway to study the impact of Bt corn on CEW. This research will provide information useful in refining long-term resistance management strategies.

EPA imposed mitigation measures, in the form of sales and distribution restrictions, as part of the requirements of registration on Bt corn hybrids that had Bt expression in silks and kernels to reduce CEW selection pressure in the South. There is a concern that CEW resistance may develop in insect populations that feed on both Bt corn and Bt cotton where Bt corn and Bt cotton/cotton acreage is in close proximity. Even though CEW only overwinter in the South, resistance would also be found in the northern states carried by migrating or wind blown adults that had emerged each spring in the South and from Mexico. Sufficient data are not yet available to indicate whether the selection pressure on CEW/CBW will be increased by large amounts of Bt corn in the South.

Grower surveys have indicated the critical need for continuing grower education before growers accept and implement of a refuge as a permanent part of a resistance management plan. Based on these surveys, growers would prefer a rotation option in which Bt crops were alternatively planted with non-Bt crops over planting Bt and conventional hybrids in a specified ratio in field to field patchworks or in-field refuges. Additional grower surveys were conducted in 1997 and

these results should be reported to the Agency in the annual reports due January 31, 1998.

Even though use of a structured refuge is accepted by all stakeholders as necessary for a long-term resistance management strategy, experts have not reached an agreement as to the size, structure, and deployment of Bt and non-Bt plots and the nature and objective of performance-monitoring activities. Agency scientists have participated in the cooperative efforts of the NC-205-led consortium. This consortium has identified critical needs for a long-term insect resistance management strategy, helped set research priorities where uncertainties existed, encouraged the adoption of national monitoring strategies and developed and disseminated educational materials to growers and the public. The NC-205 recommendations on ECB resistance management are described in NCR-602 publication, entitled “Bt-corn & European Corn Borer: Long-Term Success Through Resistance Management” (Ostlie *et al.*, 1997). The NC-205 recommendation is to have a structured refuge which is 20-30% non-Bt corn to prevent Bt delta endotoxin exposure to 20-30% of the larval populations. They also recommend that in continuous corn acreage sprayed with insecticides, the refuge size would be increased to perhaps 40% to compensate for larval mortality. In addition, a smaller refuge size may also be suitable if there are many alternate hosts providing adequate numbers of susceptible ECB. Temporal schemes involving early- and late-plantings of Bt corn interspersed with non-Bt corn may also be a viable option, but additional data are needed. Further validation of a structured block design and temporal/spatial structural design options should be encouraged.

Significant progress has been made to generate the necessary data to develop long-term resistance management strategies. Coordination of research priorities and combining of resources would more effectively lead to the development of a long-term resistance management strategy for Bt corn. EPA has fostered and participated in efforts to provide all stakeholders with opportunities for public comment on the development and implementation of long-term resistance management strategies for Bt plant-pesticides. The Agency has held Science Advisory Panel meetings (e.g., March 1, 1995 SAP Subpanel on Plant-pesticides regarding the Bt potato risk assessment and resistance management) and two public hearings (i.e., March and May 1997) on the subject of Bt plant-pesticides resistance management. The Agency plans to hold a SAP meeting in February 1998 to examine the information the Agency has gathered in efforts to develop long-term resistance management strategies for Bt plant-pesticides. In addition, other SAPs are planned to continue the Agency’s dialogue with experts and stakeholders interested in developing long-term resistance management strategies.

#### **IV. Bt cotton resistance management**

This section will discuss resistance management activities and results for 1996 and discuss any lessons learned regarding resistance management since CryI(A)c delta endotoxin expressed in cotton was first registered in 1995.

The following materials were used in preparing this section of the paper: material provided

during the public hearings, scientific publications, personal communications, 1997 Proceedings of the Beltwide Cotton Conference, EPA's Fact Sheet (U.S. EPA, 1995c), Agency review regarding pesticide resistance management, Monsanto's 1996 status reports (November 5, 1996 and February 28, 1997), literature review of the biology of the major lepidopteran pests of cotton (MRID 4404225-01), and literature review of cross-resistance potential (D227579, submission dated May 22, 1996) and USDA 1996 cotton statistics.

### *Background*

The Agency granted the conditional registration of the CryI(A)c delta endotoxin from *Bacillus thuringiensis* subspecies *kurstaki* and the genetic material necessary for its production in cotton to control TBW, CBW, and PBW in October 1995. The resistance management strategy for the CryI(A)c delta endotoxin expressed in cotton has been reviewed by the Agency prior to registration (U.S. EPA, 1995c). The conditional registration will automatically expire at midnight January 1, 2001. EPA will reevaluate the effectiveness of the registrant's resistance management plan before January 1, 2001 to determine whether to convert the registration to a registration without an expiration date. An Experimental Use Permit has been granted to Monsanto Co. and their registration submission is pending for use of a CryIIA delta endotoxin in cotton.

There are two primary resistance concerns for the registered Bt cotton: 1) development of resistance in the primary target pests, TBW, CBW, and PBW and 2) cross-resistance to the CryI(A)c and other Cry delta endotoxins expressed in other Bt plant-pesticides or Bt microbial products. The Agency concluded that to manage resistance in the long-term and to develop a long-term resistance management strategy, specific data needed to be collected on all three target pests and required such data be generated. A multi-factor resistance management plan was required to be implemented as a condition of the registration for CryI(A)c in cotton. The Bt cotton registration required a structured refugia and included grower education and training. The initial resistance management plan addressed all of the general elements of a resistance management plan. These elements included pest biology, Bt dose deployment, refugia, monitoring, effects on other susceptible nontarget lepidopteran pests, cross-resistance, integration into an IPM program, grower education and communication, and development of alternative pesticides with different modes of action. Bt cotton will not control all lepidopteran and other insect pests. For example, the Bt delta endotoxins do not control boll weevil, fall armyworm, beet armyworm, silverleaf whitefly, aphids, stink bugs, or plant (*Lygus*) bugs, all important pests of cotton. The Cry protein dose expression in the plant is considered to be a high dose for TBW, but not for CBW and perhaps not for PBW. However, the dosage in the plant is high enough to kill >80% of the CBW larvae. Therefore, the use of Bt cotton in conjunction with other IPM practices should effectively control high CBW populations and other important insect pests of cotton. These IPM practices were addressed by Monsanto in its Bollgard® cotton Grower Guide.

Because of the degree of uncertainty associated with season-long exposure of the target insect complexes to the CryI(A)c delta endotoxin, the Agency believed that before a conclusion can be

made about the potential long-term success of a resistance management strategy, additional research data, a specific monitoring plan including the development of discriminating doses for TBW, PBW, and CBW, field validation of the resistance management strategy, and annual reporting of use information and monitoring results are required. EPA required as part of registration for Monsanto: (1) to submit literature and research data on target pest biology and ecology including the data on the effectiveness of non-cotton hosts as refugia (literature review due June 1, 1996 and research data due January 31, 1998), (2) to develop a protocol for determining the likelihood of cross-resistance to other Bt endotoxins (due April 1, 1996), (3) to evaluate the potential for cross resistance (due January 31, 1998), (4) to submit a plan for a workable monitoring program (surveillance, tracking and remediation elements) (due March 1, 1996), (5) to submit an annual report of monitoring data (annually November 1 each year for preliminary results and January 31 each year for the final report for the duration of the registration), (6) to submit annual use reports (annually November 1 each year for the duration of the registration), (7) to continue development and distribution of grower education materials, (8) to continue to investigate the influence of Bt cotton on secondary lepidopteran pests, and (9) to continue to provide CryI(A)c expression information relevant to susceptibility and control of the target lepidopteran pests (due January 1, 1998). Two structured refuge options were mandated as mitigation measures. The registrant is required to submit an annual monitoring report on results and conclusions from resistance management research. Monsanto has submitted its progress report for 1996. The due dates for these data submissions are indicated in the FACT sheet (U.S. EPA, 1995c).

Two specific refuge options were mandated as requirements of registration to mitigate the development of resistance. “Option A: For every 100 acres of cotton with the Bollgard® gene planted, plant 25 acres of cotton without the Bollgard® that CAN be treated with insecticides (other than foliar B.t.k.<sup>2</sup> products) that control the tobacco budworm, cotton bollworm and pink bollworm. Option B: For every 100 acres of cotton with the Bollgard® gene planted, 4 acres of cotton without the Bollgard® gene that CANNOT be treated with acephate, amitraz, endosulfan, methomyl, profenofos, sulprofos, synthetic pyrethroids, and/or B.t.k. insecticides labelled for the control of tobacco budworm, cotton bollworm and pink bollworm. The refuge acreage must be managed similarly to the Bollgard® cotton.” In addition, if cotton with the Bollgard® gene exceeds 75% of the total amount of the cotton planted in any single country or Parish in any year, growers in that county or Parish choosing to use the 4% untreated refuge option the following year will be required to plant the 4% refuge within one mile of the respective Bollgard® cotton field. Similarly, if EPA grants a registration for cotton containing the B.t.k insect control protein to another company, EPA will determine whether the combined acreage of cotton containing the B.t.k. insect control protein exceeds 75% of the total amount of the cotton planted in a single county or Parish and inform the registrants that the 4% refuge must be planted within one mile of the respective Bollgard® cotton or other B.t.k. cotton fields.

EPA sought comment on the performance of Bt cotton in the field at two public hearings held in

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<sup>2</sup>B.t.k. = *Bacillus thuringiensis* subsp. *kurstaki*

March and May 1997. The Agency sought information regarding reported control failures for Bt cotton in 1996, possible evaluation tools concerning these failures, and the implications on future resistance management efforts. Public comments were summarized earlier in this paper in Section I. To summarize, comments received from private citizens, organic farmers and grower organizations, environmental groups, and public-interest groups indicated that the Bt cotton resistance management plan should be reevaluated. Most urged EPA to suspend the registrations of all Bt plant-pesticides and to hold a SAP meeting to reevaluate the resistance management plans for Bt cotton and Bt corn. Comments received from Monsanto, National Cotton Council, most academic/USDA scientists, and cotton farmers indicated that Bt cotton performance in 1996 was excellent. They agreed that there was no breakdown in the Bt gene technology. In general, these commenters indicated that reports of Bt cotton failure were due to an unusually high infestation of CBW in parts of the Cotton belt (south Texas, Mid-South and Southeast growing regions). Some of these infestations on Bt cotton required supplemental insecticide treatment.

As noted earlier, it is recognized that long-term resistance management will involve other IPM practices in addition to the use of Bt cotton. The three target pests, TBW, CBW, and PBW show a differential susceptibility to the CryI(A)c delta endotoxin expressed in Bt cotton. Tobacco budworm is the most sensitive of the three species to the CryI(A)c delta endotoxin. A high dose strategy exists for the TBW, but is less certain for PBW, and does not exist for CBW. The effectiveness of the high dose and refuge strategy along with other elements of the Bt cotton resistance management strategy such as adaptation to an IPM plan, scouting, and grower education, will be discussed below. Research is underway to address the areas of uncertainty in the development of a long-term resistance management strategy for Bt cotton. This paper will discuss the progress made to date to develop a long-term resistance strategy.

#### *Bt cotton acreage in 1996 and 1997*

EPA required Monsanto Co. to submit annual sales data for each state indicating the number of acres of Bt cotton planted per county as a condition of registration. Two Bt cotton varieties were available in 1996: NuCotn 33<sup>B</sup> and NuCotn 35<sup>B</sup>. About 6000 growers planted approximately 1.8 million acres of Bt cotton. Based on a three year-average, there are about 13 to 14 million cotton acres planted annually in 16 states in the U.S. Bt cotton acreage represented about 13% of the total 1996 cotton acreage planted. Most of the Bt cotton was grown in the Southeast (779,000 A) and in the Mid-South (728,000 A). Georgia (26%), Alabama (77%), and Mississippi (42%) planted the greatest number of Bt cotton acres (>300,000A/state). Fourteen Alabaman counties were planted in >75% Bt cotton acreage. Eight other counties in Florida, Georgia (2), Louisiana, Mississippi (2), North Carolina, and Texas were planted in greater than 75% Bt cotton acreage. These counties exceed the 75% Bt cotton acreage per county trigger in the registration agreement. As a consequence, in 1997, if the 4% unsprayed non-Bt refuge was employed, it should be placed within one mile of the Bt cotton in those counties exceeding the 75% Bt cotton acreage limit.

Reports indicate that production of 1.8 million acres of Bt cotton in 1996 is thought to be



responsible, in part, for reducing total insecticide applications on cotton for lepidopteran pests by 250 thousand gallons of formulated conventional insecticides. Seventy-seven percent of the total U.S. cotton acreage was infested with cotton bollworm/tobacco budworm in 1996, requiring 1.3 applications of insecticide per acre, down considerably from previous years in which 5 to 12 spray applications were used on conventional cotton to control these pests.

Nine Bt cotton varieties were available in 1997. Preliminary 1997 reports from Monsanto indicate that Bt cotton acreage has risen to about 2.2 to 2.4 million acres.

### *Analysis of Resistance Management Strategy*

#### *Dose adequacy*

The three target pests, TBW, CBW, and PBW, have a differential susceptibility to the CryI(A)c delta endotoxin. EPA recognized this fact prior to the registration of the CryI(A)c delta endotoxin produced in cotton. The tobacco budworm is the most sensitive of the three species to the CryI(A)c delta endotoxin. The levels of the CryI(A)c delta endotoxin in Bollgard® cotton are high enough in all plant parts to provide a high dose for control of TBW through the growing season. Levels of the CryI(A)c delta endotoxin do not appear high enough to support a high dose to control PBW and CBW, but PBW is more sensitive to the CryI(A)c toxin than CBW. The Agency has required further research on the effectiveness of the CryI(A)c on all three target pests.

A discussion of the 1996 performance of Bt cotton follows below for all three target pests.

#### *1) Tobacco budworm control*

All reports indicate that TBW control was excellent in 1996, although, generally, there were unusually low populations of this pest. TBW is a major economic pest of cotton. It has developed resistance to most insecticides. Bt cotton can play a critical role in the control of TBW and should lead to a reduction in the use of conventional insecticides used previously to control this pest. Bt cotton expresses the CryI(A)c at sufficiently high enough levels to be considered a high dose for control of TBW.

#### *2) Cotton bollworm control*

While the CryI(A)c expression levels are high enough to kill about 80% of all susceptible CBW, during years of high CBW infestations, the dose would not be adequate to control CBW populations below economic threshold levels during peak periods of oviposition. The Agency utilized previous reported information by Mahaffey *et al.* (1994) and Bradley (1995) that under high CBW infestation pressure, Bt cotton will not provide economic control of CBW and supplemental insecticidal sprays may be necessary. These reports show that CBW larval feeding resulted in boll damage levels as high as 32% and caused significant yield reductions when field experiments were conducted under very high CBW larval infestations. In essence, Bt cotton is not a stand-alone technology when very high cotton bollworm populations were encountered.

CBW is the least susceptible to the CryI(A)c delta endotoxin of the three target pests. All of the laboratory studies have shown that CryI(A)c, at the levels expressed in Bt cotton plants is only moderately toxic to CBW. Current estimates indicate that Bollgard®<sup>3</sup> cotton varieties, NuCotn33<sup>B</sup> and NuCotn35<sup>B</sup>, kill between 80 and 95% of the cotton bollworm larvae that feed on them. Technically, this is called a “moderate dose”, although this level provides good practical control of the pest at lower population levels. It was not expected that Bt cotton would provide a high dose to control CBW. Warnings in Monsanto’s grower guides and from extension entomologists to this effect were provided to growers. For example, Dr. Blake Layton, extension entomologist from Mississippi pointed out that producers and consultants in Mississippi were cautioned via the 1996 and 1997 Cotton Insect Control Guides, the weekly Cotton Insect Situation Newsletter, the Cotton Insect Telephone Hotline, and extension publication 2108, “Insect Scouting and Management in Bt-transgenic Cotton” that Bt cotton may require treatment in cases where high populations of CBW occurred. As a result of EPA’s analysis of the potential for resistance to develop in CBW, as well as in TBW and PBW, EPA required monitoring of the target pest populations for resistance. EPA’s analysis indicated that, in some cases, additional control measures would be necessary to reduce CBW populations below economic threshold levels. The level of CBW control in Bt cotton, the availability of other best management practices, and the pest biology were all considered as part of the development of the initial resistance management strategy both by Monsanto in its initial resistance management plan and by EPA in its evaluation of resistance risk.

In 1996, cotton bollworm populations were the highest seen in ten years in parts of the Cotton belt (i.e., Brazos Valley, Texas, Mid-South and Southeast growing regions). The lack of economic control of CBW by Bt cotton was observed by independent crop consultants, Monsanto, and others who advised affected growers to use alternative chemical controls, if necessary, as part of an overall IPM cotton growing program. In some cases, growers failed to understand the Bt cotton technology and rushed to apply insecticides when economic thresholds had not been exceeded. Nonetheless, in 1996, applications for CBW control were about 3.3 applications for non-Bt cotton and about 0.3 for Bt cotton in Mississippi. That is, it was expected that under unusually high CBW infestation pressure that supplemental insecticide treatments might be necessary and that scouting and monitoring practices and other IPM practices would have to be tailored to most effectively use Bt cotton to control insect pests. A number of other best management practices can be employed as part of a resistance management plan when using Bt cotton to control CBW. These include use of: crop rotation, structured non-Bt cotton refuges, non-Bt alternate hosts as refuge (corn, sorghum, and soybeans), alternative insecticides -- synthetic pyrethroids and new foliar spray technologies to control CBW, stalk removal, and novel Cry insecticidal genes (e.g., CryIIA). However, more documentation is needed as to their effectiveness as part of a long-term resistance management strategy.

Monsanto reported to the Agency the potential Bt cotton control failures as early as July 1996 and followed up with a full analysis of these incidents in the Fall of 1996. Monsanto performed

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<sup>3</sup> Bollgard® cotton is Monsanto’s trademark for Bt cotton varieties containing the CryI(A)c gene.

studies at all Bt cotton areas affected by high cotton infestations to determine whether cotton susceptibility to the CryI(A)c toxin had changed and whether the Bt cotton was expressing the CryI(A)c and whether the CryI(A)c expression levels and patterns had changed. Monsanto also provided the results of these studies in its 1996 annual report on resistance monitoring activities. Results of these studies indicate that there was no change in cotton bollworm susceptibility and no change in Bt expression in the Bt cotton areas affected by high cotton bollworm infestations. These studies indicated no detectable level of resistance in these populations. Unusually high infestation levels of CBW may have, in part, resulted from the dramatic increase in corn acreage in the South.

### *3) Pink bollworm control*

Reports submitted to the Agency prior to registration indicated that the CryI(A)c expression levels may not be at high dose levels for the control of pink bollworm. The Agency recognized at the time of registration that there are existing best management practices that can be used in conjunction with Bt cotton to more effectively control of PBW while making the need for a high dose requirement less critical. These best management practices include: shredding and/or plow down requirements for PBW to destroy overwintering larvae and employing late planting dates that result in early “suicide” emergence of many PBW adults. These practices plus the use of pheromone trapping and release of sterile PBW moths under a USDA/APHIS quarantine program, together with the use of Bt cotton are likely to be effective in controlling the development of resistance. However, more information is needed on whether any putative resistant adults will be temporal synchrony with susceptible insects produced in the refuge or through the use of other best management practices.

There are essentially no alternative hosts for PBW in cotton growing areas. Okra is the only known alternate host for PBW in the U.S. Research is being conducted on an effective refuge size and structure for PBW resistance management at the University of Arizona (and its collaborators). Comments provided to the Agency at the two public hearings from the National Cotton Council and University of Arizona indicate in their opinion that pink bollworm control was excellent by Bt cotton in 1996.

### *4) Other control issues*

Traditionally, TBW has been a greater concern for cotton growers than CBW because CBW resistance to conventional pesticides is limited and the cost to control CBW is relatively low compared to the cost to control TBW. In 1996, farmers in South Carolina experienced failure controlling CBW larvae with synthetic pyrethroid applications (cypermethrin resistance) (Brown *et al*, 1997). There is a concern that CBW resistance will develop to the CryI(A)c delta endotoxin which is expressed at high levels in Bt cotton over the course of full season. The major concern is that there will be added selection pressure for the development of Bt resistance as CEW moves from Bt corn to Bt cotton increasing the exposure to similar Bt delta endotoxins

expressed in both crops. This selection pressure will be especially intense if there is wide scale planting of Bt corn across the south and southeastern U.S. where Bt cotton is used and Bt microbial pesticide products are applied on other host crops of CEW/CBW. Other generations of CEW are partitioned amongst a wider number of alternate wild hosts and crops such as cotton, soybean, peanut, and vegetables as well as corn and thus the selection pressure is not as intense. However, we do not really know what proportion of CEW/CBW feed on non-cotton hosts. Planting and growing of Bt corn hybrids, expressing the CryI(A)b and CryI(A)c delta endotoxin in silks and kernels, were restricted by EPA as a mitigation measure against the development of CEW/CBW resistance (see earlier discussion under Section III).

Rapid pest adaptation resistance to an insecticide is highly dependent on the initial frequency of resistance alleles in field populations. A high dose strategy is based on the assumption that resistant alleles in field populations will be rare and occur at a gene frequency that is less than  $1 \times 10^{-3}$ . Gould *et al.* (1997) were able to directly estimate the field frequency of alleles for resistance in TBW as  $1.5 \times 10^{-3}$  by individually mating over 2,000 male TBW collected in four states to females of a Bt toxin-resistant laboratory strain, YHD2, and then screening  $F_1$  and  $F_2$  offspring for tolerance to the toxin protein. The direct estimate of the initial resistance allele frequency of  $1.5 \times 10^{-3}$  in TBW supports the  $10^{-3}$  preliminary estimate of the YHD2 resistance allele (Gould *et al.*, 1995). Genetic models indicate that a recessive allele at this frequency could lead to rapid evolution of resistant populations if Bt cotton is grown without adequate refuges (Gould, 1986; Mallet and Porter, 1992; Roush, 1994; Alstad and Andow, 1995; Tabashnik, 1994b, Liu and Tabashnik, 1997). Gould *et al.* (1997) wrote “assuming that resistance alleles are at least partially recessive and at approximately  $10^{-3}$  and the Environmental Protection Agency mandate of a 4% refuge to maintain SS moths is followed, it should be at least 10 years before Bt resistance becomes a problem in *H. virescens* populations.” This evidence for a high initial frequency of resistance alleles in TBW emphasizes the need for caution and effective resistance management at the outset of deploying Bt cotton to control TBW. Using the measured resistance allele frequency of  $1.5 \times 10^{-3}$  for TBW, predictive models can be refined and used to establish useful parameters for field experiments. Tests of resistance management tactics, such as a refuge, in the field are still needed even where there are good predictive models.

Gould *et al.* in their 1997 paper indicate that resistance could be a problem for CBW in far less time than for TBW because the CryI(A)c toxin currently deployed in cotton is not as toxic to CBW as it is to TBW. Gould *et al.* (1997) indicates that fewer than 90% of the natural, unselected larvae of CBW and ECB are killed by commercially available Bt crops. These authors indicate that if one assumes the initial frequency of a partially recessive resistance allele is  $10^{-3}$  for CBW as it has been measured for TBW, then genetic models predict that CBW populations could become resistant to the Bt cotton in 3 to 4 years even with the 4% refuge currently in use (Gould *et al.*, 1997). However, no estimates of the initial resistance allele frequency currently exist for CBW or ECB and it cannot be assumed that CEW/CBW will have the same initial resistance allele frequency as TBW. The Agency has required appropriate research on the effect of the CryI(A)c on CEW/CBW, refuge strategies, and monitoring for changes in baseline susceptibilities to all three target pests. To date, no evidence of resistance exists for any of the three target species.

## *Pest Biology*

A long-term resistance management strategy for Bt cotton is linked to understanding TBW, CBW, and PBW biology and ecology. EPA required as part of registration a literature review and additional information regarding TBW, CBW, and PBW biology and ecology. Key literature information regarding pest biology, adult movement, mating behavior, gene flow, and alternate hosts for these target pests has been reviewed and evaluated from the perspective of resistance management options for Bt cotton by Dr. Michael Caprio (Mississippi St. University) and Dr. John Benedict (Texas A & M University) for Monsanto (MRID 2204225-01, Biology of the Major Lepidopteran Pests of Cotton (June 24, 1996)). Some of this information has been useful in evaluating the effect of CryI(A)b and CryI(A)c delta endotoxins on CEW in Bt corn and its movement into Bt cotton where it is called CBW. The Agency has reviewed this information and its contents are summarized below.

### *Biology*

Larval development time is affected by a number of factors including temperature, humidity, predators and parasites. The number of generations CEW/CBW and TBW populations complete in a year varies by location. PBW prefers to oviposit directly on bolls; whereas TBW and CEW normally oviposit in the upper third of the cotton plant. Timing and temporal synchrony in adult behaviors are clearly important. Mating disruption has been an effective method for control of PBW and should be compatible with resistance management strategies for Bt cotton. The physical structure of the leaf surface (e.g., trichome density) may alter larval movement. Heliothine larvae (e.g., CEW/CBW and TBW) have the genetic potential to alter behavior in response to plant defensive compounds which may limit survival and development. Cotton with high gossypol levels hinders the development of heliothine larvae. Production of plant defensive compounds are another factor to consider with other resistance management strategies for Bt cotton. The pupal stage in heliothines is important because it is the overwintering stage that can be disrupted using cultural practices, e.g., plowing. Overwintering stage disruption should be compatible with resistance management strategies for Bt cotton. This practice is particularly useful in controlling PBW, more so than for TBW and CBW.

### *Host plant preference*

Host plant preference is useful in determining whether alternate hosts may be used as refuge sources. Both CBW and TBW have a number of alternate hosts and emerge prior to planting of their preferred hosts. The first generation in both cases utilize a variety of wild host plants. The most comprehensive review of host plant use by the CBW/TBW complex is Stadelbacher *et al.* (1986).

There are 9 different early season wild hosts of differing quality for TBW in May: *Geranium dissectum* (wild geranium), *Trifolium incarnatum* (crimson clover), *T. resupinatum* (Persian clover), *G. carolinianum* (Carolina cranesbill), *Vicia villosa* (winter vetch), *Medicago sativa* (alfalfa), *Lathyrus hirsutus* (Caley pea), *Abutilon theophrasti* (velvetleaf), and *Sicyos angulatus* (bur-cucumber). The preferred hosts for TBW are tobacco and toadflax. Corn is not a host for TBW. TBW moves to cotton in the summer and the density is about ten times less than on tobacco. Soybeans, especially late-planted soybeans, may become attractive to TBW in August, but are a less desired host than tobacco or cotton. The final generation of TBW either enters diapause in cotton fields or moves into wild hosts (e.g., beggarweed, *Desmodium tortuosum*), alfalfa, or soybeans.

As with TBW, first generation CEW/CBW primarily feeds on wild hosts during the early spring. There are five to eight wild hosts that have been noted as early season hosts: *Amaeranthus* sp., *Desmodium* spp., *Jussiaea decurrens*, *Linaria canadensis* var. *Texana*, *Solanum carolinense*, *G. dissectum*, *T. incarnatum*, and *A. theophrasti*. Alfalfa and early season wheat can also be hosts for CEW/CBW. Cultivated hosts are more attractive to second generation CEW/CBW. These hosts include peanuts, sweet pepper, strawberries, gladiolus, soybeans, cotton, okra, sweet potato, tomato, alfalfa, tobacco, geranium, lima beans, wax beans, snap beans, sorghum, and corn. The primary hosts for the second generation include tobacco, toadflax, whorl stage corn and cotton. There is a broader cultivated host range for CEW/CBW than for TBW. As corn begins to silk, it becomes very attractive to CEW/CBW. Populations in silk stage corn can reach very high densities. By the third generation of CEW/CBW, corn has begun to dry up and is not as attractive so alternate hosts including cotton, sorghum, peanuts, tomatoes, and late-planted soybeans become more important. Millet and cowpeas may also be important hosts at this time. After the third and fourth generations have survived primarily cultivated hosts, the final generation or two may move back onto wild hosts such as purselane (*Portulaca oleracea*) or alfalfa.

Although late-planted soybeans may be considered as a suitable refuge crop for cotton, they are not the primary host for either CEW/CBW or TBW. Cotton is preferred by both CEW/CBW and TBW over soybeans. Soybeans can support heliothine populations, but they will only do so when the more attractive cotton has already started to senesce. Soybean may be a better host for CEW/CBW in some areas, like North Carolina, but more research on the cotton/soybean interface is warranted. It seems unlikely that soybeans are a significant refuge crop while cotton is still blooming.

It is clear from the literature review that there are many possible alternate hosts for CEW/CBW and TBW during the season. However, the exact utilization patterns vary with climate and cultivation practices. The complexity of movement of CEW/CBW and TBW amongst various possible hosts requires more study before it is possible to determine which alternate hosts may serve as a refuge. Therefore, for now, we must rely on non-Bt cotton as the refuge.

PBW, in contrast to either CEW/CBW or TBW, is more restricted to cotton in the U.S. Populations of PBW don't increase until there are bolls. There are 36 possible alternative host plants, but the extent to which these alternative hosts sustain PBW has not been addressed. In Arizona, only okra and wild cotton act as possible alternative hosts for PBW, but these areas where okra and wild cotton grow are very small and isolated from the cotton growing areas.

### *Movement*

Research has shown that CBW and TBW are highly mobile moths, both from mark/recapture studies and from studies of genetic structure. Data suggest that CEW/CBW is a more mobile moth than TBW. It is possible to see long-distance dispersal in CEW/CBW of more than 160 km, particularly as it moves out of corn. Estimates of the genetic variability between populations predict the gene flow over long periods of time (long term movement rates). It is important to measure the distance moved before mating. Another indication of mobility is the appearance of moths in an area prior to local emergence. This is common for CEW/CBW, but not for TBW. On the other hand, intra field movement is more limited for PBW. Most flights are nocturnal and are short, less than 200 m. Genetic studies have indicated that there are low levels of population differentiation suggesting high rates of genetic exchange between populations. In contrast to CEW/CBW and TBW, PBW is much less mobile.

Because the use of refuges in resistance management depends upon the assumption of random mating between moths from refuge and treated sites and random oviposition, it is important to improve estimates of the likelihood of matings to occur at a localized sites versus those between moths from long distance and gene flow throughout the growing season.

Based on the published data, additional research efforts are needed to address larval and adult movement, mating behavior, ovipositional preferences, population dynamics, gene flow, survival and fecundity, fitness costs, and the use of alternate cultivated or wild hosts as refuges. The varied cropping systems for cotton, including local and regional differences, should be considered. This information is valuable in designing an effective refuge strategy that maximizes the probability that susceptible individuals arising from a structured refuge will find and mate with the few resistant individuals that survive exposure to the delta endotoxin produced in the Bt plant. Research data regarding these efforts must be submitted by Monsanto to the Agency by January 31, 1998.

### *Biology of Resistance*

EPA required as a condition of registration a protocol for determining the likelihood of cross resistance for the CryI(A)c delta endotoxin to other Bt endotoxins. Monsanto provided a number of published articles regarding the potential for cross-resistance and the results of a midgut

binding assays to examine receptor binding properties for CryI(A)c and CryIIA in TBW, CBW, and ECB (D227579, dated May 22, 1996). Monsanto concluded that the potential for cross resistance to develop among Bt proteins exists and that additional laboratory or field studies will not add significantly to the current body of knowledge concerning the development of cross resistance between the CryI(A)c and CryIIA delta endotoxins. Instead, Monsanto has proposed three criteria to determine the suitability of insect control proteins for insertion into plants for a multiple gene strategy: (1) use of a non-Bt protein with a distinct mode of action (when available) (2) the similarity (degree of amino acid homology) between the two protein sequences if derived from Bt; and (3) differences in the mode of action or in binding parameters if derived from Bt. Monsanto does not intend to send additional data regarding the potential development of cross resistance between the CryI(A)c and CryIIA delta endotoxins. EPA is currently reviewing Monsanto's proposal. Currently, one of the conditions of registration is for data evaluating the potential for cross resistance to develop. These data are due to EPA by January 31, 1998.

Information on the nature of resistance to Bt in TBW, CBW, and PBW are necessary to develop effective long-term resistance management for Bt cotton. Such information is useful in evaluating the potential for Bt endotoxins to be used in rotational or pyramiding schemes. Currently this work is limited to working with laboratory-selected insect populations since resistance in the field has not been detected. Laboratory studies selecting for TBW, CBW, or PBW-tolerant strains provide information on the genetic potential of each of these three target pests to develop resistance, but do not provide information on whether resistance will develop under field conditions. Bt cotton and TBW, CBW, or PBW in the field may pose a different situation than larvae feeding on Bt insecticides in a laboratory diet. Laboratory selected TBW and PBW colonies tolerant to Cry proteins exist. The CBW-tolerant colony selected against CryI(A)c at Mississippi State University no longer exists. Laboratory-selected Bt-tolerant TBW, CBW, and PBW colonies will allow experiments concerning the mode of resistance, the genetic basis for resistance, and the potential for cross resistance to other Bt toxins to be conducted. These laboratory-selected Bt-tolerant insect colonies can also be used to estimate the initial resistant allele frequencies in field populations and be used to confirm the efficacy of the estimated LC<sub>99</sub> as a discriminating dose.

#### 1. *Laboratory selection for TBW Cry-tolerant colonies and Cross-resistance.*

There is a limited knowledge on the genetics and mechanisms of resistance to CryI(A)c toxin in TBW, CBW, and PBW. Several laboratory strains of TBW have been selected for resistance to the Bt delta endotoxins (Stone *et al.*, 1989; Sims and Stone, 1991; Gould *et al.*, 1992; Gould *et al.*, 1995).

The YHD2 strain of TBW developed a very high level of resistance to CryI(A)c and related CryI endotoxins (Gould *et al.*, 1995). This colony was developed before Bt cotton was commercially deployed in 1996 and was selected on an artificial diet with an acute exposure to high amounts of purified CryI(A)c. The LC<sub>50</sub> for YHD2 was 2,000X more



resistant to the Bt endotoxin than the  $LC_{50}$  for the susceptible TBW colony. Recent experiments have shown that YHD2 can survive on Bt cotton that expresses the CryI(A)c toxin. A major portion of the resistance in the YHD2 strain is encoded by a single gene with mostly recessive inheritance. YHD2 confers resistance to CryIA toxins (a, b, and c) as well as CryIF. Research using genetic markers have indicated the location of this major resistance gene is on linkage group 9 with some evidence that there is a minor resistance gene located on linkage group 11. Biochemical analyses indicate that resistance in the YHD2 strain is associated with decreased toxin binding to the membrane of larval midgut cells, the toxin's apparent binding site and site of action. Other decreases in Bt toxin binding have also been found in Bt-resistant strains of other lepidopteran species and inheritance of the Bt resistance was at least partially or completely recessive (Van Rie *et al.*, 1990; Ferré *et al.*, 1991).

Gould *et al.* (1992) reported broad cross-resistance in another laboratory strain of TBW that was not as highly resistant as YHD2 that did not appear to be related to binding site modifications. Laboratory selections of a TBW population using CryI(A)c resulted in varying levels of cross-resistance to CryI(A)a, CryI(A)b, CryIB and CryIC insect control proteins. These laboratory data indicate that there is the potential for cross-resistance by TBW to develop to a number of Cry proteins. It is unlikely that laboratory selective procedures provide the identical selective conditions as exist in the field. The ability to select for tolerance to Cry proteins in the laboratory in different insect pests indicates that it is prudent to use appropriate resistance management strategies.

Midgut binding assays performed by Monsanto (D227579, dated May 22, 1996) examining the mode of action of CryIIA have shown that, unlike CryI and CryIIIA, neither saturable binding nor a saturable binding component was found for CryIIA on the midgut brush border of CEW/CBW, TBW, or ECB larvae. CryIIA did not dilute and block CryI(A)c binding; however, CryI(A)c effectively diluted CryIIA and stopped the initial binding of CryIIA to the brush border. These observations indicate that CryI(A)c and CryIIA share a common component for binding on the midgut brush border. These same results were reported for CEW/CBW alone by English *et al.* (1994). These authors went further to conclude that CryIIA is significantly less soluble in the digestive fluids of CEW/CBW than CryI(A)c. Third instar CEW/CBW feeding was arrested by both proteins, but acute morbidity was delayed for CryIIA. CryIIA formed voltage-dependent and not highly cation-selective channels in planar lipid bilayers unlike CryI(A)c and CryIIIA. These authors conclude that CryIIA is less bioactive against CEW/CBW than CryI(A)c, but represents a unique mode of action among the delta endotoxins. CEW/CBW is the least sensitive of three target species (i.e., TBW, CEW/CBW, and PBW) to the CryI(A)c delta endotoxin expressed in cotton. The results seem to indicate that CryIIA may be less effective than CryI(A)c in controlling CEW/CBW. However, the CryIIA delta endotoxin has a different target binding site on the midgut membrane than CryI(A)b/CryI(A)c and thus may be useful in pyramiding or stacking with other Cry genes to combat insect pest resistance.

## *2. Predicting the Evolution of CEW/CBW resistance - Computer simulation models.*

Monsanto, Dekalb, Novartis Seeds and investigators from North Carolina State University (Dr. John Van Duyn, Dr. Fred Gould, Dr. J.R. Bradley, and Dr. George Kennedy), Virginia Polytechnic Institute and State University (Dr. Ames Herbert) and from the University of Maryland (Dr. Galen Dively) are developing strategies for developing computer simulation models which predict the evolution of resistance to CryI(A)b/CryI(A)c proteins by CEW/CBW within the corn/cotton system. Additionally, research protocols are being developed for validating model assumptions and output. Research areas include: (1) Assessing the impact of Bt corn on CEW/CBW adult emergence and oviposition in cotton; (2) Contribution of alternate hosts as refuges for CEW/CBW; and (3) Impact of Bt on CEW/CBW overwintering survival and fecundity; and (4) Assessing survivorship of CEW/CBW and TBW on pollen.

### *Effective Refuges*

All available evidence supports the conclusion that a “structured” refuge is necessary to the success of a long-term resistance management strategy. Two refuge options were mandated as requirements of the Bt cotton registration to mitigate the development of resistance: 20% sprayed refuge (Option A) and 4% unsprayed refuge (Option B).

Monsanto’s 1996 year-end report indicates that compliance with the refuge requirements was excellent, >98% of those growers surveyed. Monsanto visited 2,346 of 6000 Bollgard® growers (about 40% of the total) and found that 60% used Option A (the 20% sprayed non-Bt cotton refuge) and 38% used Option B (the 4% unsprayed non-Bt cotton refuge) and 1% used both. The remaining 1% did not have an adequate refuge in place due to flooding or other circumstances. Between 82-88% of the growers surveyed had their refuges within 1 mile of the Bollgard® cotton. This means that 12-18% of the growers had refuges more than 1 mile from Bt cotton, this could be a problem.

Several entomologists discussed the subject of effective refuge size and structure in their written comments to EPA and in the published literature. Experts including Dr. Mike Caprio, Dr. Fred Gould, Dr. Rick Roush, and Dr. John Benedict have provided written comments to the Agency during the two public hearings in March and May 1997 and stated that the 4% unsprayed refuge may not be large enough to produce a relatively high number of susceptible cotton bollworm adults to mate with any resistant insects that may develop on Bt cotton with a low dose for CBW. Some growers employing this option sustained large yield losses in the refuge due to fall armyworm and CBW infestations. Resistance management analyses by EPA (September 27, 1995) indicated that the 4% unsprayed refuge was not the preferred option because of the potential for its failure, under high pest population pressure, to provide enough susceptible individuals to mate with any resistant individuals throughout the growing season. Economic considerations may also contribute to its potential lack of success. Farmers may be unwilling because of the cost involved to treat the unsprayed refuge areas in an identical fashion, agronomically, e.g., weed control, irrigation, fertilization etc. They may choose agronomically

undesirable land or place the refuge too far away from the Bollgard® cotton acreage to be of any use in resistance management.

Reports from extension entomologists in the 1997 Proceedings of the Beltwide Cotton Conferences indicated that in some cotton-growing areas, 50 to 80% yield losses were incurred in the 4% unsprayed refuge areas. Some indicate that they would not be recommending the use of the 4% unsprayed refuge in 1997 (Smith, 1997). A number of entomologists have indicated in their written comments to EPA that the lack of high dose for CBW control should be considered in designing an effective refuge strategy and the existing refuge requirements should be modified. These experts are recommending that Option B/the 4% unsprayed refuge should be discontinued or drastically expanded to perhaps, 30 to 50%. Option A/the 20% sprayed refuge should be continued or expanded.

Dr. Fred Gould (entomologist), North Carolina State University, commented that Bt cotton does not provide a high dose strategy for control of CBW and PBW. Furthermore, the current refuge options associated with Bt cotton amounted to an effective refuge size of 4% and in his estimation the effective refuge size would need to be increased to counterbalance the lack of a high dose in CBW. The 20% sprayed refuge was considered to be a 4% effective refuge. Gould indicated the effective refuge size for Bt cotton, under the current dose situation, would have to be at least 30% non-Bt cotton.

A panel of Texas A & M entomologists stated in their written comments to EPA that a 30% unsprayed refuge may be impractical or too costly to the grower. They recommend that a 20% non-Bollgard cotton sprayed refuge be planted and managed in a manner to coincide with the Bollgard® cotton so the refuge is attractive to ovipositioning CBW adults and will produce moths at the same time and in the same geographical vicinity as the Bollgard® cotton, i.e., within 1 mile of the Bollgard® cotton. They recommend that the 4% unsprayed refuge should be replaced with a 20% sprayed refuge. They explained that a 20% sprayed refuge should be effective, as long as the combined corn, sorghum and soybean acreage is planted in relatively close proximity to the Bt corn to act as a refuge, is greater than the Bollgard® cotton acreage in these Bollgard® cotton producing counties.

Dr. Mike Caprio (entomologist) from Mississippi State University commented that a delay in emergence of resistance could still exist in the absence of a high dose strategy, but the emergence would occur sooner than it would in the presence of a high dose strategy. He states that “while it is true that a high dose makes the refuge strategy much more effective, we have shown in simulations of foliar applications of Bt that even survivorship rates as high as 20% could still delay resistance 5 fold compared to the rate of resistance evaluation in the absence of the refuges.” He encourages the pyramiding of multiple genes in cotton if such genes increase mortality of cotton bollworm to delay resistance development in this pest.

Gould *et al.* (1997) measured an initial resistance allele frequency of  $1.5 \times 10^{-3}$  for TBW. Using this measured frequency, these authors caution that an effective refuge is needed at the outset to

manage the development of resistance to the CryI(A)c produced in Bt cotton. They concluded that the current refuge options mandated by EPA should be effective for at least 10 years before a Bt resistance problem might be seen in TBW populations. They commented that Bt resistance could be a problem for CBW in far less time than for TBW because the CryI(A)c toxin currently deployed in cotton is not as toxic to CBW as it is to TBW. These authors indicate that if one assumes the initial frequency of a partially recessive resistance allele is  $10^{-3}$  for CBW as it has been measured for TBW, then genetic models predict that CBW populations could become resistant to the Bt cotton in 3 to 4 years even with the 4% refuge currently in use (Gould *et al.*, 1997). However, no estimates of the initial resistance allele frequency currently exist for CBW or ECB and it cannot be assumed that CEW/CBW will have the same initial resistance allele frequency as TBW.

Tabashnik (1997) commented on Gould *et al.*'s 1997 paper. He also suggests that the odds for delaying resistance could be improved by requiring larger refuges than the 4% unsprayed or 20% sprayed refuges required for Bt cotton. The 4% unsprayed refuge may not produce enough susceptible insects throughout the growing season and the 20% sprayed refuge may suppress susceptible insects because of the effectiveness of the conventional insecticide treatments. In both cases, the refuges would be ineffective.

Recent experiments by Liu and Tabashnik (1997) suggest that a 10% structured refuge helped to maintain susceptibility of a diamondback moth to *B. thuringiensis* subsp. *aizawai*. These data provide experimental evidence to support the mathematical models that refuges can delay insect adaptation to Bt (Gould, 1988; Mallet and Porter, 1992; McGaughey and Whalon, 1992; Tabashnik 1994 a, b; Alstad and Andow, 1995).

Experts have argued that a mixed seed refuge will be ineffective for managing resistance of TBW and CBW to the CryI(A)c delta endotoxin expressed in cotton because of short-range larval movement between plants. There is a threat that TBW and CBW larvae would be exposed to sublethal doses of the Bt toxin on Bt plants and then move to non-Bt producing plants allowing selection for resistance to occur. However, PBW has very restricted larval movement and a seed mix refuge strategy is more likely to work for this pest. Furthermore, PBW has a very limited host range, essentially it feeds only on cultivated cotton in the U.S. Okra and wild cotton are alternative hosts, but these plantings are extremely small and isolated from cotton acreage in Arizona.

Monsanto is wholly or partially funding a number of research activities along with other sponsors, including USDA, to determine what constitutes effective refuges for several pests. These are summarized below.

1. “*Managing Resistance to Bt-Transgenic Plants: Greenhouse and Field Tests*” (A. M. Shelton, J. D. Tang, and E. D. Earle, Cornell University, Geneva, NY and R. Roush, University of Adelaide, Australia) Using resistant diamondback moth populations, small-scale field tests are being conducted in Bt broccoli to examine the effects on refuge size,

proximity, and effects of conventional insecticide treatment on the refuge. Results from greenhouse and larval movement data, and field data showed that the number of larvae per plant in the mixed refuge was less than the number of larvae per plant in the separate refuge. This suggests that more larvae were exposed to toxin which should have intensified selection in the mixed refuge. The level of resistance, however, was low for all refuge treatments. Immigration of native susceptibles were sufficient to dilute the resistance. Both mixed and separate refuge treatments were effective in managing resistance, but it is not known what will happen if the field studies were extended to 8 or 9 insect generations. However, these researchers recommend that a separate refuge should be used over a mixed refuge. Further field experiments on refuges will be done to study the release of greater numbers of larvae earlier in the field season and release at higher resistant allele frequencies.

2. *Evaluate resistance management strategies for PBW in Bt cotton in field tests (Tim Dennehy, University of Arizona, Tuscon, AZ; Western Cotton Research Laboratory-USDA; USDA-APHIS-PPQP, Arizona Cotton Growers Association, Cotton Incorporated, and Monsanto Co.)* A three-year, 200 acre study was begun in 1997 in Eloy, Arizona to contrast the outcomes of resistance development of PBW subjected to different Bt use strategies. Five different treatments are being studied: 1) 80% Bt cotton/20% non-Bt cotton, 2) Rotations of Bt cotton (one year) with non-Bt cotton (next year); 3) In-field refuges, i.e., mixed seed, 80% Bt cotton/20% non-Bt cotton; 4) Biologically-intensive strategy (multiple control measures, Bt cotton + pheromones + nematodes + sterile male releases); and 5) Control group, non-Bt cotton with PBW controlled with conventional insecticides. PBW populations will be collected from each treatment annually and will be placed in culture and bioassayed for susceptibility to the CryI(A)c delta endotoxin.

#### *Monitoring for Resistance --Surveillance/tracking*

As a requirement of registration, EPA required that Monsanto submit a plan for a workable monitoring program, and submit the existing data for baseline susceptibility data for TBW, PBW, and CBW by March 1, 1996. Where these data do not exist, data must be submitted which provided baseline susceptibility and discriminating doses for these pests. The monitoring plans should establish specific locations in selected states that will be monitored annually at a central laboratory location, with duplicate sample collections sent to a second lab for confirmation. Monsanto will also follow up on grower, extension specialists, or consultant reports of less than expected results or control failures for TBW, CBW, and PBW as well as for cabbage looper, soybean looper, saltmarsh caterpillar, cotton leafperforator and ECB. Monsanto will also indicate in the monitoring plans how resistance management strategies would be altered should resistance be detected. A preliminary report on monitoring must be submitted to EPA annually by November 1 each year and a final report submitted to EPA annually by January 31 each year for the duration of the condition registration. Monitoring and remedial action plans were submitted in March 1996, reviewed by the Agency in April 1996, and found to be acceptable. The purpose of monitoring is to learn whether a field control failure resulted from resistance or other factors,

other than resistance, that might inhibit the expression of the CryI(A)c delta endotoxin. It may be possible to develop monitoring techniques sensitive enough to detect early changes in resistance in pest populations before it becomes widespread. Regular surveillance by growers is essential to early detection of resistance. Monitoring activities and the monitoring and remedial action plans are summarized below.

1. *Baseline susceptibility and development of diagnostic Bt concentrations for monitoring for TBW and CBW (Dr. Hardee and Dr. L.C. Adams, USDA-ARS-SIML, Stoneville, Mississippi, Monsanto sponsorship).* Diagnostic doses for CBW and TBW have been developed over several years in insect control labs at Monsanto (Sims *et al.*, 1996). The LC<sub>99</sub> estimates for the full-length CryI(A)c protein are 6.6 µg/mL for TBW and 13322 µg/mL for CBW. Clearly, the differences in diagnostic dose concentration indicate that CBW is significantly less sensitive to the CryI(A)c protein than TBW. Sims *et al.* (1996) evaluated the growth inhibition response using the full-length CryI(A)c protein for TBW and CBW. The EC<sub>99</sub> was 0.058 µg/mL for TBW and 28.8 µg/mL for CBW. These EC<sub>99</sub> estimates are considerably lower, 114-fold less for TBW and 463-fold less for CBW, than the corresponding LC<sub>99</sub> estimates for the full-length CryI(A)c protein. That is, the larval growth inhibition response is more sensitive than the diagnostic dose response and is a reasonable starting point for detecting changes in CBW and TBW susceptibility to the Bt CryI(A)c protein. Sims *et al.* (1996) validated the concept of a diagnostic dose in combination with a larval growth inhibition assay to unambiguously separate resistant from susceptible insects using a CryI(A)c protein resistant strain of TBW and F<sub>1</sub> hybrids derived by crossing the resistant strain to a susceptible TBW strain. These data indicate that it may be hard to detect resistance with a simple LC<sub>50</sub> test or to develop a simple diagnostic mortality dose. The weight gain data are much more quantitative. A combination of the diagnostic dose and larval growth inhibition assay seems to be the most efficient means of tracking population susceptibility, especially when the assay can detect the decreased susceptibility present in resistant heterozygotes.

Monitoring efforts for CBW and TBW resistance were initiated in 1996. Twenty-three different populations of these insects were collected in Arkansas, Mississippi, Oklahoma, and Texas and subjected to field doses of MVP II Bt foliar insecticide. The CryI(A)c delta endotoxin in this foliar insecticide is the most toxicologically analogous to the CryI(A)c protein expressed in Bollgard® cotton. Monitoring results showed no shifts in baseline susceptibility levels to the CryI(A)c protein. However, there were exceptionally low populations of TBW across the Cotton Belt in 1996; therefore, data from only 3 colonies of this insect were tested. This sample size is very small and is not adequate to accurately predict whether resistance is occurring in the field. Resistance monitoring efforts were expanded and continued in 1997. Determination of threshold levels of initiating remedial action need to be developed as well as the specific programs for appropriate remedial actions. The 1997 monitoring report should be submitted to the Agency by January 31, 1998.

2. *Baseline susceptibility and development of diagnostic Bt concentration for monitoring for PBW.* (Dr. Alan C. Bartlett, Western Cotton Research Laboratory, USDA/ARS, Phoenix, AZ; Dr. Tim Dennehy, Dept. of Entomology, University of Arizona, Tuscon, AZ; Dr. Larry Antilla, Arizona cotton Research and Protection Copuncil, Tempe, AZ, Monsanto sponsorship). Twenty-five percent of Arizona's cotton acreage was planted with Bt cotton in 1996. The key target of Bt cotton expressing the CryI(A)c delta endotoxin in Arizona is PBW. Baseline susceptibility to the CryI(A)c delta endotoxin was determined from five PBW populations in five Arizona counties (i.e., Stanfield, Yuma, Buckeye, Parker, and Marana). Newly hatched larvae from these populations were subjected to artificial diets containing doses of a purified solution of Bt CryI(A)c delta endotoxin provided by Monsanto. None of the neonate larvae were able to reach maturity when the CryI(A)c dose exceeded 0.005 µg/mL of diet (Bartlett, *et al.*, 1997). Baseline monitoring studies were expanded in 1997. Preliminary results will be available in 1998.

### 3. *Surveillance and remedial action.*

As noted earlier, Monsanto is required as part of the Bt cotton registration to have a workable monitoring plan and a remedial action plan. Monsanto is required to instruct customers to contact them (e.g., toll-free customer service number) if incidents of unexpected levels of TBW, CBW, and PBW damage occur as well as unexpected damage by cabbage looper, soybean looper, saltmarsh caterpillar, cotton leafperforator and ECB. Monsanto is required to report to EPA suspected incidents of resistance to these three pests. Monsanto will investigate and identify the cause for this damage by local field sampling of the plant tissue and suspect insect populations, followed by appropriate *in vitro* and *in planta* assay. Any confirmed incidents of resistance are required to be reported to EPA. If resistance is confirmed then appropriate remedial action is required to mitigate resistance. Remedial actions include: informing customers and extension agents in the affected areas of resistance problems, implementing alternative means to reduce or control the resistant populations, increasing monitoring in the affected areas, modifying refuges in the affected areas, and ceasing of sales in the affected and bordering counties. In its Bollgard® Grower Guide, Monsanto has instructed its customers to have regular surveillance programs and report any unexpected levels of TBW, CBW, and PBW damage to them and to their local extension agents. EPA considers that industry cooperation with extension and academics entomologists and consultants to be important in communicating specific information of definitions of "unexpected damage" and appropriate remedial action.

#### 1) *TBW and CBW*

In 1996, Monsanto investigated claims of Bt cotton failure in the Brazos River bottoms in East Texas and reported this information to the EPA immediately in July 1996. Monsanto investigated these "failures" at the affected sites. CBW and Bt cotton tissue were collected from high infestation areas. CBW susceptibility and Bt expression in Bt cotton

areas affected by high cotton infestations were determined. There was no change in cotton bollworm susceptibility or in Bt expression in these areas. These studies showed no detectable level of resistance in CBW populations collected in the affected areas. Experts agree that the Bt cotton performed as expected under high infestation conditions of CBW. Reports indicate that CBW populations were at the highest level measured in a decade. Bollgard® cotton killed greater than 80% of these hatching CBW, but survivors exceeded the economic threshold for control.

The situation unfolded as follows. As corn began to senesce after producing two generations of CEW, nearby cotton acreage experienced extremely heavy CBW infestations, especially in areas with high corn acreage. In many cases, these CBW larvae were able to survive by feeding on pollen material and then moving to bolls lower in the plant canopy where expression of the CryI(A)c protein is lowest. Dr. Blake Layton, extension entomologist from Mississippi, reported that the percent of CBW damaged bolls was considerably lower in the Bt due to larvae feeding on pollen in blooms and then moving to bolls rather than in the terminal region of the plant. These CBW larvae escaped detection because scouting techniques for conventional cotton normally are for the top 6" of the plant canopy. Coupled with the natural tolerance of CBW to the CryI(A)c protein compared to TBW, it is likely that a proportion of the population survived on pollen and grew large enough to tolerate higher levels of the CryI(A)c protein in other tissues. Supplemental insecticide sprays to control CBW on Bt cotton were used in some instances, but not all Bt cotton acreage was treated or needed to be treated. The results of these investigations were presented verbally to EPA at a meeting held on September 11, 1996. The formal results of the investigations were presented to EPA in written form in Monsanto's preliminary monitoring report dated November 5, 1996 and its final annual monitoring report dated February 28, 1997. EPA agrees with the findings as presented by Monsanto in these reports.

## 2) PBW

While the focus has been on the control of the TBW/CBW complex in the majority of cotton growing areas located from Texas eastward, PBW is the major target insect of Bt cotton in Arizona, California, and New Mexico cotton-growing areas. In addition to Monsanto's required efforts to respond to putative reports of resistance, a multi-agency Rapid Response Team consisting of the University of Arizona, Cotton Research and Protection Council, and headed by the Arizona Cotton Growers Association has been organized to promptly and rigorously investigate growers' claims of failure of Bt cotton to control PBW in Arizona. Putatively resistant populations will be put into culture and tested for susceptibility to Bt toxin.

### *Development of products with alternative modes of action*

Industry is developing other cotton lines that involve the expression of novel Bt genes acting by



mechanisms different from currently registered Bt genes. These novel genes could be combined with currently registered Bt genes that could be combined with existing Bt genes, insecticidal genes with mechanisms of action different from Bt, and inherent host plant resistance traits as a means for combating the development of TBW, CBW, or PBW resistance to the Cry(A)c delta endotoxin expressed in cotton. An EUP has already been granted for the CryIIA delta endotoxin expressed in cotton and the registration submission is under review. This delta endotoxin operates by a voltage-dependent mechanism. Pyramiding or stacking genes is advocated by entomologists as a powerful tool to mitigate the development of resistance. Roush (1994) has modeled the effects of pyramiding and results indicate that resistance may be delayed by greater than 1000-fold. Dr. Dave Ferro (University of Massachusetts) and Dr. Fred Gould (North Carolina State University) both provided comments encouraging the pyramiding of multiple genes in cotton if such genes increase mortality of cotton bollworm to delay resistance development in this pest. Monsanto is conducting research on the effects of CryI(A)c and CryIIA gene combinations on efficacy and resistance management. They also have ongoing efforts to discover non-Bt genes to control TBW, CBW, and PBW among other insect pests. There are also many novel non-Bt conventional insecticide products with different modes of action available to cotton growers to control the TBW/CBW complex, e.g., Tracer, Pirate, Intrepid, and Proclaim. The availability of many control options with different modes of action to control the TBW/CBW complex will help to reduce the reliance on any one control option.

### *Grower Education*

As a requirement of registration, Monsanto was required to continue the development and distribution of grower education materials including: instructions on the appropriate use of Bollgard® cotton in a resistance management program, monitoring, and reporting of resistance. Monsanto has extensive grower activities, e.g., educational seminars, brochures, video tapes, cassettes etc. Monsanto supports a number of research activities on resistance management (discussed above). Monsanto has had involved discussions with growers, crop consultants and USDA researchers and extension specialists to develop information on resistance management and integrated pest management. This information was included in the Bollgard® cotton Grower Guide.

Monsanto reported to EPA that it made personal grower surveys in 1996 and 1997 to quantify grower experience with Bollgard® cotton. The 1996 sample included 89 growers. Bollgard® cotton growers reported an average yield improvement of 6% to 16% depending on the cotton-growing region. Taking into account total insecticide system control costs and yield, they saw an economic advantage of \$33 per acre from using Bollgard® cotton even after paying the \$32 technology fee. Planting of Bollgard® cotton resulted in the elimination of the equivalent of a 250 thousand gallons of formulated insecticide products in the U.S.

Based on Bt cotton performance in 1996, academics, Monsanto, and the National Cotton Council noted that there could be improvements made in communication on CBW control in Bt cotton with the growers, general public, and consultants. The National Cotton Council indicated that

scouting practices had previously focused on the top six inches of the plant. As a result of observations made during the 1996 Bt cotton growing season, including the outbreak of CBW in the Brazos Valley in Texas, modified scouting practices in Bt cotton will be employed to examine the whole plants especially during peak bloom periods. It is currently recommended that growers continue to scout their crop on a regular basis by inspecting the entire plant, including blooms and bloom tags, to obtain an accurate larval count and damage assessment. Monsanto, growers, crop consultants, USDA researchers and extension specialists are working together to continue to develop information on how to best scout Bollgard® cotton. Their collective scouting recommendations are reflected in the 1997 Grower Guide. The 1997 Grower Guide states that “scouting at least twice per week is recommended during periods of heavy or sustained egg lay, especially during peak bloom. Scout the entire plant, including blooms and bloom tags. Larvae greater than 1/4 inch (2- 4 days old) are generally recognized as survivors that will be difficult to control with Bollgard® alone. Apply remedial insecticides if the frequency of advanced stage larvae or plant damage warrants treatment. Consult your local university and extension service for advice on thresholds appropriate for your area. Changes to these recommendations may be required under unique circumstances; consult your local crop advisor.”

Reports from extension entomologists and consultants in the 1997 Proceedings of the Beltwide Cotton Conferences indicate that effective and timely scouting is extremely important for Bollgard® cotton. Based on the 1996 Bt cotton performance, there would be an extra cost for scouting Bollgard® cotton because of the higher monitoring frequency, the more exacting and different monitoring requirements (not overreacting to egg or to the tiny first-stage larvae, judging what constitutes a second-stage larva, monitoring for stink bugs, fall armyworms etc.) compared with conventional non-Bt cotton. These higher costs may cause some growers not to use Bollgard® cotton. Additional training and labor requirements in effectively monitoring Bollgard® cotton are needed in the short term until Bt cotton scouting and treatment practices become more routine. Pest surveys should be modified and economic thresholds may be more difficult to determine in Bollgard® cotton.

Consultants and entomologists report in the 1997 Proceedings of the Beltwide Cotton Conferences that Bollgard® cotton is more valuable to the grower after boll weevil eradication programs because the CryI(A)c delta endotoxin is not effective against boll weevil. Insecticidal treatment for boll weevils will be required in areas in which they have not been eradicated. However, treating for boll weevil will also suppress the beneficial insect populations allowing other secondary pest populations to surge which then may need insecticidal treatment. Therefore, if Bollgard® cotton is used in areas that have not undergone boll weevil eradication, then strategies to ensure the preservation of beneficial insects especially later in the season will be important to help manage non-lepidopteran pests, because beneficial insects will be destroyed during early season treatment for boll weevil.

Reports indicate the 1.8 million acres of Bollgard® cotton grown in 1996 is thought to be responsible, in part, for reducing total formulated chemical insecticide applications by 250 thousand gallons. Seventy-seven percent of the U.S. cotton acreage was infested with

CEW/CBW complex in 1996. On average, there were 1.3 applications of insecticide sprays per acre where Bollgard® cotton was grown. This represents a significant reduction from previous years in which 5 to 12 applications were used on conventional cotton to control CEW/CBW populations.

Cotton consultants had little practical experience with Bt cotton prior to its wide-scale commercialization in 1996. Experiences in 1996 were shared via the Consultants Instant Information Network (CIIN). Lack of experience may have caused the costs of pest control to be higher in some instances. If so, greater familiarity with specific scouting and monitoring practices for Bollgard® cotton should lower these costs. Some consultants in the Mid-South have raised concerns about whether Bollgard® cotton is well suited for the Mid-South cotton production areas (Farr *et al.*, 1997). They are interested in several issues: whether new Bt cotton varieties will perform better agronomically than the standard non-Bt cotton lines, will they perform better in controlling CBW than the standard non-Bt cotton lines, will the Mid-South generate more boll weevil adults to overwinter out of Bt cotton fields, will resistance increase more rapidly in Bt cotton due to full season production of the toxin in the plant, and will plant bugs increase in Bt cotton.

The most important consideration for growers is whether the Bollgard® cotton will provide a greater economic value than use of conventional cotton. Comments by Texas A & M entomologist, Dr. John Benedict, and other entomologists have noted that Bollgard® cotton is not useful to all cotton producers, but is of value to those spending more than \$40 per acre for control of TBW, PBW or mixed populations of CBW, TBW or PBW on their cotton. It allows those farmers to use their equipment for other farm functions, reduces hazards of pesticide exposure and poisoning to the grower, farm employees, farm families, and consumers. Bt cotton will also be useful to improve the grower's ability to fully use IPM, to allow beneficial arthropods to be more effective, and to provide greater likelihood of relief from past crop losses, and costs of unsuccessful attempts to control insecticide-resistant TBW.

### *Public Comments*

Of the 100 comments received as a result of the two public hearing held on Bt plant-pesticide resistance management this year, about 15 specifically focused on Bt cotton resistance management issues. These comments came from academic and extension entomologists, grower groups, trade organizations, environmental groups, and industry/seed companies/trade organizations. The Agency sought information regarding reported control failures for Bt cotton in 1996, suggested evaluation tools concerning these failures, and implications on future resistance management efforts at two public hearing held in March and May 1997. These comments are summarized above in Section I of this paper.

In general, comments received from private citizens, organic farmers and grower organizations, environmental groups, and public-interest groups indicated that the Bt cotton resistance management plan should be reevaluated and that a SAP should be held. Experts from industry,

academia (almost all), and USDA noted in their comments that Bt cotton performance was excellent for control of TBW, CBW, and PBW. In 1996, following reports of Bt cotton failures in the Brazos Valley area of Texas and some other areas in the Mid-South, Monsanto tested for cotton bollworm susceptibility and Bt expression in Bt cotton areas affected by high cotton bollworm infestations. They found no change in CBW susceptibility to the CryI(A)c delta endotoxin and in Bt expression levels in the plants as compared to the baseline susceptibility levels for these locations. That is, these studies showed no detectable level of resistance in the CBW populations. Comments indicated that additional data need to be gathered to develop a long-term resistance management strategy. Progress in these research areas was summarized above.

### *Summary*

It is recognized that long-term resistance management will involve other IPM practices in addition to the use of Bt cotton. The number of insecticide applications and the type of insecticides used vary widely in Bt cotton and non-Bt cotton. Based on the 1996 Bt cotton performance, there would be an extra cost for scouting Bt cotton because of the higher monitoring frequency, the more exacting and different monitoring requirements (not overreacting to egg or to the tiny first-stage larvae, judging what constitutes a second-stage larva, monitoring for stink bugs, fall armyworms etc.) compared with conventional non-Bt cotton. Additional training and labor requirements in effectively monitoring Bt cotton appear to be needed in the short term until these practices become more routine. Pest surveys will have to be modified and economic thresholds may be more difficult to determine in Bt cotton. Best management practices should be tailored specifically for Bt cotton. A unified, multi-stakeholder effort to determine research priorities and develop a long-term resistance management strategy is essential.

The three target pests, TBW, CBW, and PBW show a differential susceptibility to the CryI(A)c delta endotoxin expressed in Bt cotton. Tobacco budworm is the most sensitive of the three species to the CryI(A)c delta endotoxin. A high dose strategy exists in Bt cotton for the TBW, but the existence of a high dose strategy is less certain for PBW, and does not exist for CBW. In 1996, there was an unusually high infestation of CBW in the Cotton belt. Monsanto's research to determine cotton susceptibility and Bt expression in the Bt cotton areas affected by high cotton bollworm infestation showed no detectable level of resistance in these populations. That is, there was no change in cotton bollworm susceptibility or Bt expression levels in the affected areas as compared to previous levels. Reports in the published literature, from Monsanto, and EPA's analysis recognized the fact that Bt cotton would not produce a high dose to control CBW and that supplemental insecticide treatment might be necessary. Supplemental insecticide treatment was necessary in some cases in 1996 because of high CBW populations, but not on all Bt cotton acreage. In 1996, overall insecticide use dropped on cotton by about 250 thousand gallons, in part, because of the introduction of Bt cotton.

EPA concluded that to develop a long-term resistance management strategy and manage resistance effectively, specific data needs were made requirements of the Bt cotton registration: (1) target and secondary pest biology and ecology, (2) cross-resistance potential, (3) monitoring

data (baseline susceptibility and discriminating dose determination), (4) effect of Bt cotton on secondary lepidopteran pest (e.g., cabbage looper, soybean looper, saltmarsh caterpillar, cotton leaf perforator, and ECB), and (5) expression. EPA also required annual monitoring and annual use reports. EPA mandated specific refugia requirements and grower compliance has been high. EPA required continued development and distribution of grower education materials.

An extensive literature review regarding pest biology, host-preference, and movement were provided by Monsanto and reviewed by EPA. However to develop a long-term resistance management strategy, research efforts appear to be needed to address larval and adult movement, mating behavior, ovipositional preferences, population dynamics, gene flow, survival and fecundity, fitness costs, and the use of alternate cultivated or wild hosts as refuges. Currently, one of the requirements of the registration agreement is for further evaluation of the target pest biology. These data must be submitted to EPA by January 31, 1998.

Literature regarding the potential for cross-resistance to develop between CryI(A)c and CryIIA and a protocol for evaluating the cross-resistance between CryI(A)c, CryIIA and other Bt toxins were submitted by Monsanto. Monsanto concluded that the potential for cross-resistance amongst Cry proteins exists and that additional data and field studies will not add significantly to the current body of knowledge concerning the development of cross-resistance between the CryI(A)c and CryIIA delta endotoxins. Data presented indicated that CryI(A)c and CryIIA operate by a different biochemical mechanism of action. Monsanto does not intend to send additional data regarding the potential development of cross resistance between the CryI(A)c and CryIIA delta endotoxins. EPA is currently reviewing Monsanto's proposal. Current registration requirements require additional data evaluating the cross-resistance potential to be submitted by January 31, 1998.

Data on the genetics and mechanism of resistance to Bt in TBW, CBW, and PBW are useful to measure the effectiveness of a long-term resistance management strategy for Bt cotton. Such information is useful in evaluating which Bt endotoxins could be used effectively in a rotational or pyramiding scheme. In the absence of field resistance in any of the three target pests, laboratory-selected tolerant colonies are needed to examine the mechanism of resistance, the genetic basis for resistance, and the potential for cross-resistance. These laboratory-selected colonies can also be used to estimate the initial resistant allele frequencies in field populations and be used to confirm the efficacy of the estimated  $LC_{99}$  as a discriminating dose. A number of registrants are collaborating with academic entomologists to develop computer simulation models which predict the evolution of resistance to CryI(A)b/CryI(A)c proteins by CEW/CBW within the corn/cotton system.

As a requirement of registration, Monsanto must provide annual reports on its monitoring activities. Monsanto submitted a monitoring and remedial action plan in 1996. EPA accepted these plans. The 1996 results of its monitoring program include the development of baseline susceptibility data and discriminating dose concentrations for each of the three primary target pests. Baseline susceptibility and development of diagnostic Bt concentration for monitoring have

been developed for TBW and PBW and are under development for CBW. Validation of these diagnostic doses for detecting susceptibility changes in Bt cotton fields is needed. Baseline monitoring studies for TBW/CBW and PBW were expanded in 1997. No changes in susceptibility to the CryI(A)c delta endotoxin were detected in the tested populations of TBW, CBW, and PBW. The monitoring programs for TBW, CBW and PBW were expanded in 1997. Threshold levels for remedial action still need to be developed as well as the remedial action themselves.

Two structured refuge options were mandated by EPA as mitigation measures: Option A, a 20% sprayed refuge and Option B, a 4% unsprayed refuge. Monsanto's 1996 annual report indicates that there was a high compliance with the refuge requirements amongst the growers surveyed. Sixty percent used Option A and 38% used Option B. A number of entomologists have indicated that the lack of a high dose for CBW control was not considered in models predicting the effectiveness of the refuge strategy and that the existing refuge requirements should be modified for Bt cotton. One option discussed by these experts was to discontinue the 4% unsprayed non-Bt cotton refuge because it may not produce enough susceptible insects to mate with putative resistant insects that emerge from Bt cotton fields throughout the growing season. These experts believe that the 20% sprayed non-Bt cotton refuge should be continued or expanded. They indicate that the sprayed refuge should be planted within one mile of the Bt cotton and that it should be managed in the same way as the Bt cotton. The refuge must be temporally and agronomically equal to the Bt cotton so that it produces susceptible insects at the appropriate time and place to mate with resistant insects produced on the Bt cotton. One recommendation was that the size of the refuge be expanded to 30% unsprayed non-Bt cotton acreage. However, such an increase in size of untreated acreage may not be economically attractive for growers. Yield considerations and cost of maintenance of the refuge acreage are important considerations for the adoption of a particular refuge option.

Entomologists suggest that a seed mix refuge strategy may work for PBW because of its limited larval movement. A seed mix refuge strategy to control PBW resistance development is being field tested over the next 3 years in Arizona.

In conclusion, a great deal of scientific data have been gathered during the first two years of commercialization of Bt cotton. Progress has been made toward the development of a long-term resistance management strategy. Additional scientific data are still required as part of the Bt cotton registration: (1) target and secondary pest biology and ecology, (2) cross-resistance potential, (3) monitoring data (baseline susceptibility and discriminating dose determination), (4) effect of Bt cotton on secondary lepidopteran pest (e.g., cabbage looper, soybean looper, saltmarsh caterpillar, cotton leaf perforator, and ECB), and (5) expression. EPA also required annual monitoring and annual use reports. EPA mandated specific refugia requirements and grower compliance has been high. EPA required continued development and distribution of grower education materials. Grower and consultant education is vital to the success of any long-term resistance management strategy. The high dose and refuge strategy should be integrated into a set of best management practices specific for Bt cotton. These practices include: use of

biological control, appropriate use of conventional insecticides to control CBW, application of pheromone for mating disruption, sterile insect release, and stalk destruction programs for PBW; application of new selective foliar sprays that kill Bt resistant and susceptible pests while preserving beneficial insects to clean up target pests that escape the insecticide, deployment of effective refuges to manage resistance, and crop rotation.

## V. Document Summary

This paper has provided a review of the public hearing comments and an update on the status of the resistance management plans in Bt potato, Bt corn, and Bt cotton. EPA is holding a Science Advisory Panel meeting in February 1998 to obtain input from resistance management experts regarding this information and the development of long-term resistance management strategies for Bt potato, Bt corn, Bt cotton and other Bt crops. The Agency plans to host additional SAP meetings to continue this evaluation process.

Since Bt plant-pesticides became commercially available in 1996, growers have adopted this technology as part of their IPM practices to control pests in potato, corn, and cotton. Based on industry reports sent to EPA, the greatest adoption of Bt crop technology has been by cotton growers, especially in the southeastern United States in 1996, with about 13% of the cotton acreage, 1.8 million acres, and an estimated 2.2 to 2.4 million acres in 1997 planted in Bt cotton. Corn growers planted in Bt corn about 400,000 acres in 30 states in 1996, but are expected to have planted an estimated 4 million acres in 1997. Potato growers planted about 10,000 acres of Bt potato in 1996 and an estimated 25,000 acres in 1997. The differences in the rate of adoption of Bt potato, Bt corn, and Bt cotton are likely due, in part, to the availability of effective alternatives, the cost of the biotechnology crop, extent of regional pest problems, and familiarity and acceptance of the technology by growers. For example, there are several insecticide alternatives for Colorado potato beetle control. The cost of the technology and familiarity with the technology may have discouraged a wider adoption by corn growers. The adoption rate for Bt cotton was especially high for a new technology because few, if any, effective alternatives existed to control tobacco budworm in cotton especially where resistance to registered conventional pesticides was extremely high in states such as Mississippi and Alabama.

No evidence exists that resistance to Bt Cry proteins produced in transgenic potato, corn, or cotton has developed in the 1996 or 1997 growing season. Monitoring for susceptibility changes to the different registered Cry proteins, CryI(A)b, CryI(A)c, and CryIIIA, has been conducted for Colorado potato beetle, European corn borer, tobacco budworm, cotton bollworm, and pink bollworm. Baseline susceptibility studies show a wide-range of variability, so it is important to look at susceptibility changes in the context of the baseline range for a particular geographic location of the pest (i.e., different portions of a State). No changes in baseline susceptibility have been detected for any of the target insects exposed to the Cry proteins expressed in Bt potato, Bt corn, and Bt cotton. This information indicates that there has been no measured increase in tolerance to date to the Cry proteins expressed in Bt crops. However, current monitoring methods may not be sensitive enough to detect changes in susceptibility to the toxins.

Laboratory-tolerant colonies of Colorado potato beetle, European corn borer, tobacco budworm, and pink bollworm have been created through selection against purified Cry proteins or mixtures of Cry proteins using Bt microbial pesticides. Laboratory colonies tolerant to high levels of Cry proteins do not exist for all target pests, e.g., CEW/CBW. The ability of insects to develop high levels of tolerance to Bt in the laboratory indicates that these insects possess the genetic potential



to develop resistance to Cry delta endotoxins expressed as Bt plant-pesticides. It is unlikely that laboratory selective procedures provide the identical selective conditions as exist in the field. The ability to select for tolerance to Cry proteins in the laboratory in different insect pests indicates that it is prudent to use appropriate resistance management strategies.

The CBW outbreak beginning in East Texas in 1996 has not been shown to be due to CBW resistance to the CryI(A)c delta endotoxin expressed in Bt cotton, but due to extremely high infestation levels of the insect and the lower sensitivity this insect has to the CryI(A)c delta endotoxin relative to TBW and PBW. Scouting detected the CBW lower in the plant canopy of Bt cotton than expected and, in some cases, supplemental chemical insecticides were used to control CBW. The fact that supplemental insecticides might be necessary to control unusually high CBW infestations was not unexpected and was considered in the Agency's review of the initial resistance management strategy for Bt cotton. Modifications to the CBW scouting program for Bt cotton were made for the 1997 season to improve detection of the CBW larvae which might escape the Bt delta endotoxin by feeding on blooms and bloom tags that are lower in the cotton plant.

Most cotton growers complied with the structured refuge requirements. Cotton growers seem to prefer the 20% sprayed refuge option (Option B) which allows them to treat the refuge with chemical insecticides normally used to control TBW, CBW, and PBW (except for Bt microbial pesticides). This option appears to more reliably provide a higher yield in the refuge acreage than the 4% unsprayed refuge option (Option A) which often had higher management costs and lower yields. Most cotton researchers who commented at the two public hearings favored the 20% structure refuge as a better strategy for Bt cotton resistance management because this refuge is more likely to provide a greater percentage of susceptible insects throughout the growing season to mate with any rare resistant individuals that might survive in the Bt cotton fields. EPA received comments that the 4% unsprayed refuge was decimated early in the growing season so there were few, if any, adult moths surviving to mate with any resistant insects that survived in the Bt cotton fields.

EPA believed that during the first five years following commercialization (approximate time-limit of the conditional registrations for Bt corn, there would not be enough Bt corn acreage to provide substantial Bt selection pressure for the development of ECB resistance. Consequently, EPA did not mandate specific refuge requirements for Bt corn, but EPA has required research data on the size, structure, and deployment of a structured refuge. A combination of temporal and structured refuges are being studied. A draft refuge strategy must be submitted to the Agency by August 1998 and a final refuge strategy is required to be submitted by January 1999. Implementation of an EPA-approved structured refuge plan or an EPA-approved alternative resistance plan is required no later than April 1, 2001. Monsanto and Dekalb are requiring structured refuges as part of grower agreements. Beginning in the 1998 growing season, Novartis Seeds has adopted the NC-205 consortium's recommendations published in NCR-602 publication entitled "Bt Corn & European Corn Borer - Long Term Success Through Resistance Management" (Ostlie *et al.*, 1997). The NC-205 recommendation is to have a structured refuge which is 20-30% non-Bt corn

to prevent Bt delta endotoxin exposure to 20-30% of the larval populations. They also recommended that in continuous corn acreage sprayed with insecticides, the refuge size would be increased to perhaps 40% to compensate for larval mortality. In addition, a smaller refuge size may also be suitable if there are many alternate hosts providing adequate numbers of susceptible ECB. Mycogen has not made any specific refuge recommendations in its Grower Guide, but is supportive of the use of refuges and supportive of the NC-205 recommendations.

Monsanto/Naturemark requires a structured refuge as part of grower agreements for use of Bt potato. EPA has required that Monsanto mandate specific refuge requirements as a condition of registration for Bt cotton. Monsanto has implemented these refuge requirements through a grower agreement. Research is underway to study whether the seed mix option is viable for PBW resistance management. EPA is encouraged by reports of the high level of compliance with structured refuge in Bt cotton and Bt potato and by the tremendous reduction in the use of conventional insecticides that has resulted from adoption of Bt cotton.

A great deal of research is underway to study the elements that are necessary for long-term resistance management strategies for Bt potato, Bt corn, and Bt cotton. Specific research data were required as part of the Bt corn and Bt cotton conditional registrations and was recommended for the Bt potato registration. These data included: the dosage effectiveness on the target pest(s), monitoring data including baseline susceptibility and validation of the diagnostic dose concentration, pest biology and ecology, influence of the Bt crop on secondary lepidopteran pests, the impact of CryI(A)b/CryI(A)c produced in Bt corn on the selection of CEW/CBW resistance in Bt corn and Bt cotton, impact of Bt on CEW overwintering survival and fecundity, effective refuges, alternate hosts as refuges, and cross-resistance potential. Additionally, alternative pest control strategies and integration into existing IPM programs are being examined for each of the Bt plant-pesticides. All of these data will provide the basis for specific improvements to the existing resistance management strategies. Future information is especially important for understanding the selection of CEW/CBW resistance in Bt corn and Bt cotton especially in the southern United States because CEW/CBW usually moves from silking corn to cotton, has multiple generations per year, and overwinters in the South. Exposure to Cry delta endotoxins produced in both Bt corn and Bt cotton in two or more generations a year could rapidly accelerate development of resistance to Cry delta endotoxins. Research results and predictive models studying this situation are expected to be submitted to the Agency in 1998.

## Appendix 1: Table of Pest Acronyms

Acronym	Common Name	Scientific Name	Crop
CBW	Cotton Bollworm	<i>Helicoverpa zea</i> (Boddie)	cotton
CEW	Corn Ear Worm	<i>Helicoverpa zea</i> (Boddie)	corn
CPB	Colorado Potato Beetle	<i>Leptinotarsa decemlineata</i> (Say)	potato
ECB	European Corn Borer	<i>Ostrinia nubilalis</i> (Huebner)	corn
FAW	Fall Armyworm	<i>Spodoptera frugiperda</i> (J. E. Smith)	corn
PBW	Pink Bollworm	<i>Pectinophora gossypiella</i> (Saunders)	cotton
SCSB	Southern Corn Stalk Borer	<i>Diatraea crambidoides</i> (Grote)	corn
SWCB	Southwestern Corn Borer	<i>Diatraea grandiosella</i> (Dyar)	corn
TBW	Tobacco Budworm	<i>Heliothis virescens</i> (Fabricius)	cotton

**Appendix 2: Table of Registered Bt Plant-Pesticides**

<b>Events/Products</b>	<b>Toxin</b>	<b>Crop</b>	<b>Company(s)</b>
NewLeaf®	Cry IIIA	Potato	NatureMark/ Monsanto
176	Cry I(A)b	Corn	Ciba/Novartis Seeds, Mycogen Corp.
BT11	Cry I(A)b	Corn	Northrup- King/Novartis Seeds
MON 801	Cry I(A)b	Corn	Monsanto
MON 810	Cry I(A)b	Corn	Monsanto
DBT418	Cry I(A)c	Corn	DEKALB Genetics Corp.
Bollgard®	Cry I(A)c	Cotton	Monsanto

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